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CONTENTS

Continued on back cover

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Analysis of RAPD DNA profiles between the normal and sex-limited breeds of mulberry silkworm, *Bombyx mori*

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ABSTRACT: Sex-limited breeds have great significance in sericulture industry for efficient management of hybrid layings production, which involves separation of sexes of parental breeds. Sex limited breed of Pure Mysore is characterized by larval markings in females while the males are plain, which is evolved by translocation of the larval marking gene (p) of 2nd chromosome onto W chromosome. CSR2 Sex-limited breed is a productive bivoltine breed with females spinning yellow cocoons, while the male cocoons are white, where the yellow blood gene (Y) from the 2nd chromosome is translocated onto the W chromosome. With the objective of detecting the polymorphism in the DNA profiles of normal and sex-limited breeds of these two breeds, the present study was undertaken using 18 RAPD decamer primers. Among the breeds, 112 discrete bands ranging from 200–2400 bp in size were detected, with an average of 6.22 bands per primer. The overall polymorphism between PM and CSR2 were 77.6%. PCR products specific to both normal and sex-limited breeds have been detected. Comparison of genetic similarity among the selected breeds showed the highest similarity index value between males and females of PM (N) breeds and the lowest between CSR2 (SL) females and PM (SL) males. Cluster analysis grouped the breeds, clearly separating multivoltine from bivoltine breeds.

KEYWORDS: mulberry silkworm, sex-limited breed, RAPD, polymorphism

INTRODUCTION

Separation of males and females is a very important task during the preparation of hybrid eggs and bulk of the silk in India is produced from the crossbreed of polyvoltine females x bivoltine males. Hence during the preparation of the crossbreeds, sexes are identified at the pupal stage based on the external markings on the abdomen, which involves cutting open of every cocoon. Half of the cut cocoons involving

polyvoltine males and bivoltine females are wasted as they cannot be reeled. To overcome the difficulties, to reduce the manpower required for sex separation and to avoid the wastage of cut cocoons, efforts were made during the early 1940s by Japanese scientists for developing sex-limited or autosexing breeds. A number of dominant genes for the manifestation of various characters in the egg, larva, pupa, cocoon and moth stages are located on the autosomes. Further, it is well established that the sex chromosome mechanism in silkworm is of ZZ in male and ZW in female, with W chromosome having a strong female determining potency. If a dominant gene is translocated to the W chromosome, the dominant gene is expected to be transmitted only to the female progeny, as there is no crossing over in females. As a result, the character is expressed only in females. Thus the sexes can be easily separated without much labour.

This principle was used by Tazima in 1941 and he succeeded in translocating the dominant sable marking gene (p^{Sa}) located on the 2nd chromosome to the W chromosome. Thus in 1944, the first sex limited breed was authorized in Japan for commercial use. Thereafter, several sex limited breeds were developed using genes for larval markings, cocoon colour and egg colours (Kimura *et al.*, 1971; Mano, 1984; Yamamoto, 1989).

In India also efforts were made to develop sex limited strains. Sengupta (1968) transferred the larval markings of Saniish 18, a Russian sex limited race to J122 and C110 stocks. Scientists of Breeding laboratory at CSRTI, Mysore have evolved sex limited breeds for larval markings in Pure Mysore, MY1, AP1 (SLA) races (Nagaraju *et al.*, 1989). These sex limited breeds are having the larval marking gene at the 'p' locus from the 2nd chromosome translocated onto the W chromosome. Recently, sex limited breeds for cocoon colour, namely, CSR2 (SL) and NANDI have been evolved where the female cocoons are yellow and the male cocoons are white in colour, which can be easily separated by farmers at the cocoon stage for preparation of hybrids (Basavaraja *et al.*, 2003, 2004). In case of sex-limited breed for cocoon colour, the yellow colour of the cocoon is due to the presence of the dominant yellow cocoon gene 'C' in the 12th chromosome as well as the dominant yellow blood gene 'Y' in the 2nd chromosome. Hence both 'C' and 'Y' genes are required for expression of the cocoon colour. As the gene 'Y' is translocated to the W chromosome in the sex-limited breed, only the females will show the yellow cocoon, while, the male cocoons are white due to the absence of the 'Y' gene In these breeds.

A wide array of DNA marker techniques like RFLP, RAPD, AFLP, Microsatellites, STS, etc., are available for genetic studies and have been proved useful in silkworm genome analysis and for the detection of polymorphism (Nagaraju and Goldsmith, 2002). RAPD markers are well suited for genetic mapping, for plant and animal breeding applications, and for DNA fingerprinting, with particular utility for studies of population genetics. RAPD markers can also provide an efficient assay for polymorphisms, which should allow rapid identification and isolation of chromosome—specific DNA fragments. Genetic stocks carrying deletions or additions of large chromosomal segments could be screened relative to appropriate controls, to identify the

regions of the genome carrying the deletion or addition. Genetic mapping using RAPD markers has several advantages over other methods: (i) a universal set of primers can be used for genomic analysis in a wide variety of species, (ii) no preliminary work, such as isolation of cloned DNA probes, preparation of filters for hybridizations, or nucleotide sequencing, is required. The most significant advantage of this method is that the determination of genotype can be automated. Genetic maps consisting of RAPD markers can be obtained more efficiently, and with greater marker density, than by RFLP or targeted PCR-based methods (Williams *et al.*, 1990).

In the present study, the DNA profiles of the normal and sex-limited strains of Pure Mysore (larval marking) and CSR2 (cocoon colour) races have been analysed using RAPD markers with the objective to compare the allelic variations in the RAPD profiles among the normal and sex-limited breeds.

MATERIALS AND METHODS

Silkworm stocks used

In the present investigation, four silkworm stocks comprising Pure Mysore (Normal—PM N) , Pure Mysore (Sex-limited—PM SL), CSR2 (Normal—N) and CSR2 (Sex-limited—SL) were used. PM is an indigenous polyvoltine race used in south India for the last two hundred years. In this breed both female and male larvae are plain. PM (SL) was evolved in 1987 using a polyvoltine stock AP1(SL) as donor parent, where, all female larvae are marked while the males are plain. The CSR2 (Nl) breed was evolved during the 1990s through extraction from the highly productive Japanese hybrids, which was subsequently acclimatized to the Indian tropical condition and fixed by following appropriate breeding protocols. In case of CSR2 (Nl), both female and male cocoons are bright white. CSR2 (SL) was evolved in 2003 by using CC1(SL), a sex-limited bivoltine breed for cocoon colour and CSR2 (Normal) by transferring the translocated W chromosome containing the 'Yellow Blood 'gene from a Japanese breed into CSR2 (N). This breed is characterized by the yellow coloured female cocoons and white coloured male cocoons and hence is called 'Sex-limited 'breed. The characteristics of the breeds used are indicated in Table 1.

Genomic DNA extraction

Genomic DNA of PM (N), PM(SL), CSR2(N) and CSR2(SL) (Sex-wise) were extracted from whole bodies of fifth instar larvae (3d day), after removing midgut and haemolymph and purified as per Nguu *et al.* (2005). Concentration and purity of extracted DNA was determined spectrophotometrically at 260 nm and 280 nm. They were further diluted to a final concentration of 20 ng/μl of Tris-EDTA buffer (5 mM Tris, 0.5 mM EDTA, pH 8.0) for RAPD analysis.

Primers used, PCR amplification and electrophoresis

18 RAPD primers (OPA 1 to 20 except OPA 6 and OPA14) purchased from Operon Technologies, Inc., Alameda, USA) were used to amplify the template DNA from

TABLE 1. Characteristics of the silkworm breeds used

Characteristics	Pure Mysore (Normal)	Pure Mysore (Sex-limited)	CSR2 (Normal)	CSR2 (Sex-limited)
Larval marking	Plain	Marked female	Plain, bluish white	Plain, bluish white ♂
		Plain male	♂and ♀	Yellowish white ♀
Cocoon colour & shape	Light greenish yellow spindle	Light greenish yellow spindle	Bright white, oval	Bright white, oval ♂Golden yellow, oval ♀
Cocoon grains	No grains	No grains	Fine to medium	Fine to medium
Pupation rate (%)	88–90	85–88	85–90	85–90
Cocoon shell (%)	14–15	14–15	24–26	21–22
Filament length (m)	350–375	350–375	1000–1100	1000–1100
Filament size (Denier)	1.6–1.8	1.6–1.8	3.0–3.1	2.5–3.0
Raw silk %	8–9	8–9	19–20	14–16
Neatness (points)	76–80	6–80	85–90	85–90
Reelability (%)	73–75	73–75	80–85	80–85

the silkworm breeds used. The details of RAPD primers used is given in Table 2. PCR reactions were carried in a PTC 200 gradient thermal cycler (MJ Research, USA) using the RAPD primers. Amplifications were carried out in 25 μl reactions containing 1 × reaction buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0), 1.5 mM $MgCl_2$, 6–8 picomoles of primer, 200 μM dNTPs, 2 U Taq DNA polymerase and 20 ng of template DNA. A negative control was set up omitting the DNA from the reaction mixture. The reaction mixture was pre-heated at 95 °C for three minutes followed by 40 cycles (94 °C for 3 min, 40 °C for 1.30 min and 72 °C for 2 min). A final extension for 10 minutes at 72 °C was given after the completion of the cycles. Resulting PCR products were electrophorectically analyzed through 1.5% agarose gels containing ethidium bromide (5 μg/ml) in1 × TAE buffer (pH 8.0) and documented in the gel documentation system (Syngene, U.K.). A molecular weight marker (lambda with EcoR1/Hind III double digest) was used for the analyses of the fragment size. All amplification reactions were carried out at least thrice in order to make sure consistency and repeatability of fingerprints generated using selected RAPD primers.

Data analysis

For analysis, RAPD fragments were treated as independent and unweighted characters and a binary matrix was produced whereby the presence or absence of each DNA fragment for each sample was recorded 1 or 0 , respectively. Faint or poorly amplified

TABLE 2. Details of RAPD primers used

Primer Code	Primer Sequence (5' to 3')	Molecular Weight (bp)
OPA-01	CAGGCCCTTC	2964
OPA-02	TGCCGAGCTG	3044
OPA-03	AGTCAGCCAC	2997
OPA-04	AATCGGGCTG	3068
OPA-05	AGGGGTCTTG	3099
OPA-06	GGTCCCTGAC	3004
OPA-07	GAAACGGGTG	3117
OPA-08	GTGACGTAGG	3108
OPA-09	GGGTAACGCC	3053
OPA-10	GTGATCGCAG	3068
OPA-11	CAATCGCCGT	2958
OPA-12	TCGGCGATAG	3068
OPA-13	CAGCACCCAC	2942
OPA-14	TCTGTGCTGG	3050
OPA-15	TTCCGAACCC	2948
OPA-16	AGCCAGCGAA	3046
OPA-17	GACCGCTTGT	3019
OPA-18	AGGTGACCGT	3068
OPA-19	CAAACGTCGG	3037
OPA-20	GTTGCGATCC	3019

fragments were excluded from the analysis. From the binary matrix, the total number of RAPD fragments, breed-specific and polymorphic bands were calculated for each primer. The breed-specific markers are those RAPD bands that are exclusive only to the specific breed for a given primer.

A Dice similarity matrix was generated using the equation $S = 2Nxy/(Nx + Ny)$, where Nxy is the number shared markers between 'X' entry and 'y' entries. Nx is the total number of markers in 'x' entry and Ny is the total number of markers in 'y' entry. Genetic distance (1-s) was calculated and a dendrogram was constructed based on the similarity matrix data set by applying un-weighted pair group method of arithmetic averages (UPGMA) using the NTSYS-pc analytical software (Rohlf,1998).

RESULTS

Polymorphism between PM and CSR2 breeds

A total of 112 reliable amplified fragments were detected using 18 Operon primers which ranged in size from 200–2400 bp, using standard RAPD-PCR amplification protocol followed in our laboratory. The number of amplicons ranged from 2 (OPA 12) to 12 (OPA 17) in Pure Mysore and CSR2 breeds with an average of 6.22 markers per primer (Table 3). The number of polymorphic bands observed between PM and CSR2 breeds were 87, out of the 112 bands analysed with a polymorphism of 77.6%.

TABLE 3. Polymorphism observed among the four silkworm breeds

Sl. No	RAPD primer	No. of bands scored	No. of mono-morphic bands	No. of poly-morphic bands	% of polylmorphism
1	OPA 01	7	1	6	85.7
2	OPA 02	7	5	2	28.6
3	OPA 03	6	0	6	100.0
4	OPA 04	4	0	4	100.0
5	OPA 05	8	4	4	50.0
6	OPA 07	6	1	5	83.3
7	OPA 08	7	1	6	85.7
8	OPA 09	10	5	5	50.0
9	OPA 10	3	0	3	100.0
10	OPA 11	6	3	3	50.0
11	OPA 12	2	0	2	100.0
12	OPA 13	5	0	5	100.0
13	OPA 15	7	0	7	100.0
14	OPA 16	4	0	4	100.0
15	OPA 17	12	2	10	83.3
16	OPA 18	5	2	3	60.0
17	OPA 19	9	0	9	100.0
18	OPA 20	4	1	3	75.0
	Total	**112**	**25**	**87**	**77.6**

Polymorphism between Normal and SL breeds of PM and CSR2

Out of 112 amplified products which ranged from 208 bp to 2361 bp, 102 were monomorphic (91%) and 10 were only polymorphic(9%) between PM(N) and PM(SL) breeds. Only with one primer, namely, OPA 15, 100% polymorphism was observed. In case of CSR2 (N) and CSR2(SL), the product size ranged from 60–2696 bp, of which 79 were monomorphic (70.5%) and 33 were polymorphic (29.5%). OPA 10, OPA 12, OPA13 and OPA 16 showed 100% polymorphism between the normal and sex-limited breeds of CSR2. Thus, CSR2 showed higher % of polymorphism when compared to PM (Table 4).

Breed specific RAPD products detected

Among the 18 primers screened, 19 PM specific amplified products were detected with 10 primers. Further, 22 bands specific to CSR2 were amplified by 10 primers (Table 5). Two PCR products of 1744 bp (OPA2) and 2242 bp (OPA8) were found only in case of PM (SL) breed, which was absent in other breeds. However, we could not observe any bands which were specific to PM(N) breed. Interestingly, 5 primers generated 6 RAPD amplicons which were detected only in CSR2 (SL) breed, while, 5 bands were found to be specific to CSR2(N) breed amplified by 4 primers.

TABLE 4. Polymorphism observed among Pure Mysore and CSR2 breeds

Sl. No.	RAPD primer	Between PM (SL) and PM (N)				Between CSR2 SL and CSR2 (N)			
		1	2	3	4	1	2	3	4
1	OPA 01	7	7	0	0	7	6	1	14.3
2	OPA 02	7	5	2	28.6	7	7	0	0
3	OPA 03	6	6	0	0	6	3	3	50.0
4	OPA 04	4	4	0	0	4	2	2	50.0
5	OPA 05	8	8	0	0	8	6	2	25.0
6	OPA 07	6	6	0	0	6	5	1	16.7
7	OPA 08	7	6	1	14.3	7	7	0	0
8	OPA 09	10	0	0	0	10	10	0	0
9	OPA 10	3	3	0	0	3	0	3	100.0
10	OPA 11	6	6	0	0	6	4	2	33.3
11	OPA 12	2	2	0	0	2	0	2	100.0
12	OPA 13	5	5	0	0	5	0	5	100.0
13	OPA 15	7	0	7	100.0	7	7	0	0
14	OPA 16	4	4	0	0	4	0	4	100.0
15	OPA 17	12	12	0	0	12	12	0	0
16	OPA 18	5	5	0	0	5	4	1	20.0
17	OPA 19	9	9	0	0	9	2	7	77.8
18	OPA 20	4	4	0	0	4	4	0	0
	Total	**112**	**102**	**10**	**8.9**	**112**	**79**	**33**	**29.5**

1 = Total No. of bands, 2 = No. of monomorphic bands,
3 = No. of polymorphic bands, 4 = % of polymorphism

Genetic similarity among silkworm breeds used

Pair-wise comparison of RAPD bands scored among the silkworm breeds showed the highest similarity value (0.986) has been observed between males and females of PM (N) breeds, followed by 0.980 observed between PM (SL) female and PM (N) female. Similarly, the highest similarity of 0.936 was found between CSR2(SL) female and SL male and the next highest value of 0.877 was observed between CSR2(SL) male and CSR2(N) female. Further, the lowest value of 0.478 was noticed between CSR2 (SL) female and PM (SL) male, followed by 0.507 between CSR2(N) male and PM (SL) male (Table 6). The cluster analysis based on UPGMA method based on similarity index values has generated the dendrogram which has clearly separated the PM and CSR2 breeds into two groups (Fig. 2).

DISCUSSION

Utility of RAPD markers for analyses of polymorphism between diapausing and non-diapausing breeds that represents a high degree of divergence with respect to geographical origin, quantitative and biochemical characters are described by various authors from time to time (Nagaraja and Nagaraju, 1995; Nagaraju and Singh, 1997; Nagaraju *et al.*, 2001; Chatterjee and Pradeep, 2003). Recent studies conducted

TABLE 5. Breed specific RAPD products detected

Primer	PM Specific	PM SL specific	PM Nor. specific	CSR2 specific	CSR2 SL specific	CSR2 Nor. specific
OPA 1	1945 bp			986 bp		2117 bp
	1584 bp			831 bp		
OPA 2		1744 bp				
OPA 3	1427 bp			831 bp		
				698 bp		
OPA 4				947 bp	889 bp	918 bp
OPA 5	653 bp			475 bp		
OPA 7	2328 bp			564 bp	947 bp	
	1480 bp					
	208 bp					
OPA 8	1204 bp	2242 bp		1118 bp		
	372 bp			698 bp		
				84 bp		
OPA 9	1118 bp			947 bp		
	831 bp			564 bp		
				576 bp		
OPA 10					831 bp	1375 bp
						631 bp
OPA 11				1427 bp		
OPA 12	441 bp				412 bp	
OPA 17	2361 bp			2695 bp		
	1800 bp			1867 bp		
	416 bp			764 bp		
	292 bp			358 bp		
				176 bp		
				60 bp		
OPA 19	1584 bp				889 bp	276 bp
	947 bp				228 bp	
OPA 20	1033 bp			1204 bp		
				564 bp		

at CSRTI, Mysore (Betterson, 2006) clearly discriminated breeds producing oval cocoons (CSR2 and CSR3) and dumb-bell cocoons (CSR5) using RAPDs. It may be mentioned here that oval breeds are basically of Chinese origin and dumb-bells are from Japanese source.

Our present investigation has clearly indicated potential of RAPD markers for the analysis of polymorphism existing among different breeds of silkworms. Nineteen PCR products amplified with 10 primers were found to be specific to PM breed which were absent in CSR2. Further 22 amplified products of the same 10 primers found only in CSR2 breed which were not detected in PM. The overall polymorphism between PM and CSR2 were found to be 77.6%. In this context, it is pertinent to note that Pure Mysore is a traditional south Indian polyvoltine breed characterized with shorter life span, higher resistance to temperature and diseases, and lower productivity traits with yellow cocoons. On the contrary, CSR2 is endowed with longer life span, lower

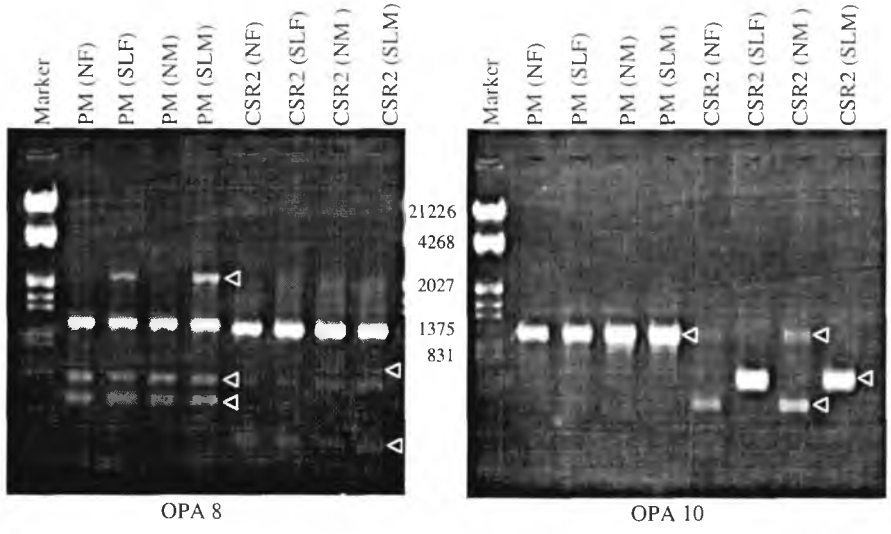

FIGURE 1. Representative DNA profiles amplified with OPA 8 and OPA 10 RAPD primers. Arrows indicate normal specific and SL specific RAPD bands

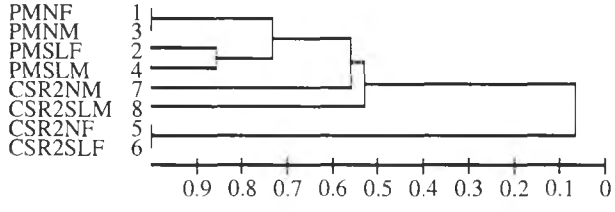

FIGURE 2. Dendogram generated by genetic similarity matrices based on RAPD profiles in the normal and sex-limited breeds

resistance to temperature and diseases and higher productivity traits with white oval cocoons, which is evolved from the Japanese hybrids (Mal Reddy *et al.*, 2005). Thus, they are genetically highly divergent showing contrasting features which is supported by our observation of high degree of polymorphism in the RAPD profiles.

Out of the 112 discrete bands generated between the sex-limited breeds(PMSL) and normal breeds of PM (PM N) when amplified with 18 primers, polymorphic bands were generated by only three primers. OPA15 showed 100% polymorphism followed by OPA 2 (28.6%) and OPA 8 (14.3%), and the average polymorphism between PM(SL) and PM(N) was found to be only 8.9%. PM (SL) evolved by Nagaraju *et al.* (1989) is characterized with larval markings in the females whereas the normal breeds are plain. Apart from this phenotypic difference, all quantitative traits are on par with the normal breeds. Hence there might not be marked polymorphism in DNA level which is confirmed by our results. However, when the same eighteen primers were

used for amplification and analysis, four primers viz. OPA10, OPA12, OPA13 and OPA16 produced amplicons which showed 100% polymorphism between CSR2(SL) and CSR2(N) breeds and the overall polymorphism was found to be 28.8%. CSR2 (SL) female breeds are characterized by yellow haemolymph spinning yellow cocoons whereas the CSR2(N) spin white cocoons. This phenotypic difference in cocoon colour might be responsible for the higher level of polymorphism in the RAPD profiles found between CSR2(N) and CSR2(SL) breeds.

The similarity index values among the males and females of PM (N) as well as PM (SL) were found to be very high ranging from 0.931 to 0.986, indicating their close genetic relationship, with a morphological difference just by the expression of larval marking genes located on the p locus of the translocated 2 chromosomal fragment to the W chromosome in PM(SL) female. However, among the males and females of CSR2(N) and CSR2(SL) the values showed a wider range from 0.806 to 0.936, indicating the possibility of higher genetic dissimilarity of the donor parent used for evolving the sex-limited breed. The lowest similarity value of 0.478 found between CSR2(SL) female and PM (SL) male clearly indicates the wide genetic divergence existing between the non-diapausing PM and diapausing CSR2 breeds. The dendrogram generated based on similarity index data has clearly grouped PM and CSR2 breeds separately as expected due to their higher genetic distance. Further, both sexes of PM(N) and PM(SL) breeds are clustered in two sub-groups.

During the course of our investigation, we were able to detect amplicons clearly demarcating SL breeds from normals both in PM and CSR2 selected for this study. Two PCR products of 1744 bp (OPA2) and 2242(OPA8) were found to be present in both males and females of PM (SL) breed, whereas they were absent in both the sexes of PM(N) breeds. Likewise four amplicons of 889 bp (OPA4), 947 bp (OPA 7), 831 bp (OPA 10) and 412 bp (OPA 12) were detected in males and females of CSR2 (SL) breed, and not in their normal ones.

However, the sex-limited breed specific bands appeared in the DNA profiles of PM (SL) and CSR2(SL) discriminating them from their normal breeds, cannot be considered as markers linked to the 2nd chromosomal fragment translocated onto the W chromosome, as these markers were present in males too. Appearance of these markers specific to SL breeds might have been inherited from their parental source. Comparison of DNA profiles of the parents (CC1(SL) and CSR2 (N) and their progeny CSR2(SL) (males and females) using the same primers may confirm the origin of these amplicons. The detachment analysis of the translocated W chromosome of another sex-limited breed p(B) of *Bombyx mori* conducted by Yokoyama *et al.* (2003) showed that one female specific RAPD marker identified by them was amplified from the region of the second chromosome fragment which was attached to W. Hence, there is a need to compare the DNA profiles of Normal and sex-limited breeds with more number of RAPD primers to detect the markers linked to chromosome segments attached to the W chromosome.

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Optimization of *in vivo* mass production of HpNPV in teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae)

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ABSTRACT: *In vivo* mass production technique for HpNPV in the homologous host, *Hyblaea puera* was optimized. The criteria for optimization included Occlusion Body (OB) yield and harvest percentage with reference to larval stage, dosage, incubation period and incubation temperature. Under optimum condition, fourth instar larvae infected with a dose of 10^5 OBs $larva^{-1}$ and incubated for 72 h at 25 ± 2 °C yielded a mean maximum of 3.3×10^9 OBs. This accounts for a 33,000 fold increase over inoculated dose. The yield obtained is 6.87 times more than the earlier production record of this NPV in *H. puera* larvae.

KEYWORDS: *Hyblaea puera* , HpNPV, Nucleopolyhedrovirus , mass production, teak defoliator

INTRODUCTION

Insect pathogenic baculoviruses offer a means of pest control that is environmentally safe and which can be produced locally. At present the only viable option for large-scale production of baculovirus is *in vivo* replication of the virus in the homologous host (Cherry *et al.*, 1997). The paper deals with the *in vivo* mass production of nucleopolyhedrovirus (NPV) of the teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae), a major pest of teak. *Hyblaea puera* NPV (HpNPV) is considered to be the most promising biocontrol agent against the teak defoliator which is capable of inflicting an estimated annual loss of 44 per cent of the wood volume increment in teak (Nair *et al.*, 1985).

In vivo mass production of HpNPV had been attempted earlier using both field collected as well as laboratory reared *H. puera* larvae (Sajeev *et al.*, 2005). However, the highest virus yield for field collected and laboratory reared fifth instar larvae were 3.6×10^8 OBs (Occlusion bodies) and 4.8×10^8OBs respectively. Since it is known that yield as well as cost determines the viability of using an NPV as biocontrol

agent, it becomes imperative to look into the optimum host stage, inoculum dosage, incubation period and microclimate. Here the focus is on the work done to optimize the techniques for mass-producing *Hp*NPV using laboratory reared *H. puera* larvae.

MATERIALS AND METHODS

Test insects

The *H. puera* larvae for the study were obtained from the Entomology Laboratory at Kerala Forest Research Institute (KFRI) Subcentre Nilambur, Kerala, India, which had been reared over 50 cycles. The larvae were maintained on a semisynthetic diet standardized earlier (Nair *et al.*, 1998). The larvae used in the study were within a narrow weight range: third instar (9–13 mg), fourth instar (26–37 mg) and fifth instar (80–110 mg).

HpNPV inoculum preparation

The HpNPV used in the study was obtained from a stock culture maintained in the HpNPV mass production unit of KFRI Subcentre, Nilambur. The virus multiplication and isolation was carried out as per standard method (Biji *et al.*, 2006). From the isolated HpNPV, aliquots were prepared (decimal series from 10^2 to 10^8 OBs per ml) by serial dilution using distilled water.

Bioassay procedure

HpNPV inoculums prepared from the stock solution were presented to the test larvae on individual teak leaf disc (0.5 cm^2 area) prepared from tender teak leaf collected from teak nursery, using a 0.5–10 μl micropipette. The inoculum over the leaf disc was allowed to dry for a period of 10 min. A single larva was placed in each rearing tube containing treated leaf disc. The larvae that consumed the whole leaf disc within 6 h period were selected for the study and transferred to individual rearing tubes containing virus free semi synthetic diet. Larvae fed on leaf discs treated with water served as control.

To quantify virus production, 10 larval rearing tubes were sampled at every 12 h interval till 120 h p.i. and the harvested larvae were frozen to death. Number and weight of the larvae harvested for each time point was recorded. The larvae of each lot were triturated separately using tissue homogeniser and the homogenate was made up to known volumes with distilled water. Enumeration was carried out using improved Neubauer's haemocytometer. Three replicates were maintained per dosage per larval instar. The test larvae were maintained at 26 °C and RH of 60 per cent and examined daily for virus death. The selection of the better combination of the larval stage, dosage and incubation period including the optimal temperature regime was based on the OB yield per unit diet. OB yield per unit diet for a specific time point was calculated as OB yield per larva × percentage of harvested larvae/100.

For determining the optimum temperature regime for HpNPV mass production,

fourth instar larvae within a narrow weight range (27 to 36 mg) were used. Cohorts of 10 larvae were fed with 10^4, 10^5 and 10^6 OBs per larva, presented on leaf discs. After 6 h of inoculation, the treated larval cohorts were incubated at 15, 25 and 35 ± 2 °C temperature regimes under a 12L: 12D photoperiod. Control larvae were placed at each of the above temperature regimes. Larval mortality was recorded at every 12 h interval and the OB yield per larva was estimated by enumerating the larval homogenate using improved Neubauer's hemocytometer.

Data analysis

Under optimization of mass production aspects, the significance of variation with respect to OB yield per unit diet for three different stages against different doses and different incubation periods were analyzed by LSD. An attempt was made to predict the OB yield per unit diet for individual larval stages by depicting a multiple linear regression model using dose, incubation period and their interactive effects. To test the significance of variation between the OB yield per unit diet obtained for different temperature regimes LSD was performed. The analyses were performed using a computer specific software package: SPSS for windows (Std. Version 10.01).

RESULTS

Percentage of larvae retrieved

The percentage of infected larvae retrieved through time course of infection for all the three larval stages tested are presented in Fig. 1. Retrieval rate of infected larvae varied with incubation period, dose and stage of larvae. In the case of third instar larvae, cent per cent harvest was registered during the early periods of incubation (till 60 h p.i.) against lower doses (10^2–10^4 OBs per larva). At higher doses ($10^5$and 10^6 OBs per larva) the retrieval curve fell slowly in the initial hours of incubation (from 12 h p.i. onwards) and deepened towards the end of the incubation period. In the case of fourth and fifth instar larvae, the retrieval percentage showed a decreasing trend towards the end of the incubation period in all the doses tested. However, the retrieval percentage in successive doses for the same incubation period was found to increase in contrast with that of third instar larvae.

OB yield per unit diet

In an efficient mass production system achieving maximum number of larvae harvested is equally important as to obtain maximum virus production per larva. Hence for practical mass production, "mean OB yield per unit diet" was arrived at, considering both the OB yield per larva and harvest percentage. The mean OB yield per unit diet obtained for the three larval stages (third to fifth instar) against different doses of HpNPV is presented in Table 1. The mean OB yield per unit diet varied with the larval stage, dose ingested and also with the incubation period. The maximum mean OB yield obtained for different doses ingested was within 48–72 h p.i. for third instar larvae, between 72 and 84 h p.i. for fourth instar larvae, and between 84 and 96 h

TABLE 1. Mean OB yield per unit diet (± S.E. values) against different doses for three larval stages

Larval stages	Ingested dose	OB yield per unit diet ($\times 10^7$) at different hours post inoculation									
		12	24	36	48	60	72	84	96	108	120
III instar	10^2	0.49 ± 0.03	2.10 ± 0.10	2.30 ± 0.05	3.70 ± 0.20	6.70 ± 0.20	14.40 ± 0.40	3.36 ± 0.05	–	–	–
	10^3	0.80 ± 0.05	1.20 ± 0.04	2.40 ± 0.05	3.20 ± 0.20	3.40 ± 0.10	6.35 ± 0.90	4.00 ± 0.50	–	–	–
	10^4	0.95 ± 0.03	1.90 ± 0.05	2.00 ± 0.20	4.40 ± 0.30	8.00 ± 0.20	1.10 ± 0.08	0.40 ± 0.08	–	–	–
	10^5	1.00 ± 0.08	1.58 ± 0.08	2.08 ± 0.08	3.85 ± 0.10	4.80 ± 0.20	0.92 ± 0.05	–	–	–	–
	10^6	0.90 ± 0.60	0.88 ± 0.08	2.10 ± 0.20	4.50 ± 0.03	0.53 ± 0.10	–	–	–	–	–
IV instar	10^2	1.38 ± 0.05	2.15 ± 0.20	3.40 ± 0.20	10.10 ± 0.40	39.20 ± 0.05	43.00 ± 0.00	49.77 ± 0.05	39.78 ± 0.04	22.56 ± 0.05	4.65 ± 0.20
	10^3	3.30 ± 0.10	10.00 ± 0.90	18.00 ± 0.50	25.00 ± 0.00	35.00 ± 0.03	58.50 ± 0.05	62.00 ± 0.30	57.20 ± 0.20	21.00 ± 0.20	6.62 ± 0.08
	10^4	3.00 ± 0.08	14.00 ± 0.00	30.00 ± 0.08	60.00 ± 0.08	70.00 ± 0.08	77.00 ± 0.10	101.50 ± 0.20	75.00 ± 0.02	44.00 ± 0.00	24.50 ± 0.20
	10^5	7.00 ± 0.08	30.00 ± 0.20	70.00 ± 0.20	85.00 ± 1.00	218.89 ± 1.00	328.30 ± 5.00	228.00 ± 3.00	77.50 ± 8.00	36.00 ± 0.50	20.00 ± 0.50
	10^6	3.50 ± 2.00	10.00 ± 0.20	45.00 ± 0.50	65.00 ± 3.00	200.00 ± 10.00	319.50 ± 6.00	101.50 ± 1.00	72.00 ± 2.00	32.00 ± 2.00	38.00 ± 2.00
V instar	10^2	2.90 ± 2.00	5.00 ± 0.10	6.00 ± 0.10	11.50 ± 0.10	12.83 ± 0.20	19.89 ± 0.03	23.36 ± 0.09	22.75 ± 0.06	3.90 ± 0.06	2.16 ± 0.06
	10^3	3.50 ± 0.06	6.50 ± 0.30	8.00 ± 0.50	10.00 ± 0.20	19.50 ± 1.00	32.00 ± 0.50	34.92 ± 0.70	48.00 ± 0.90	14.70 ± 0.60	12.00 ± 0.70
	10^4	2.00 ± 0.90	8.00 ± 0.20	10.00 ± 0.50	13.50 ± 0.50	43.00 ± 0.50	53.00 ± 4.00	57.00 ± 4.00	74.70 ± 6.00	50.96 ± 4.00	25.62 ± 1.00
	10^5	2.00 ± 1.00	3.00 ± 0.30	13.00 ± 0.20	17.50 ± 0.50	35.20 ± 0.50	58.50 ± 0.60	89.00 ± 0.50	66.00 ± 0.00	42.75 ± 0.90	16.20 ± 0.80
	10^6	5 (0.2)	9.5 (0)	10 (0.5)	15 (0.5)	60 (0.8)	100 (5)	232 (3)	178 (2)	60 (8)	18.05 (5)

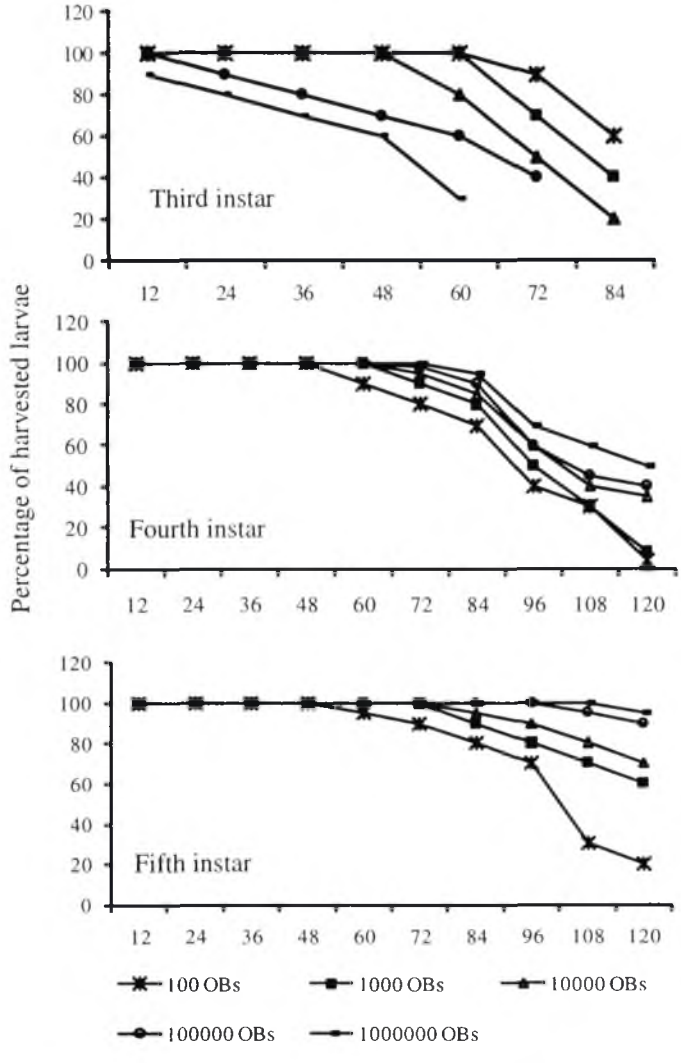

FIGURE 1. Interactive influence of host larval stage, HpNPV inoculum dose and incubation period on larval harvest

p.i. for fifth instar larvae. The maximum mean OB yield per unit diet against different doses of HpNPV tested against three larval instars is given in Table 2. Here, the mean maximum OB yield per unit diet (3.3×10^9 OBs), was obtained from the fourth instar larva fed with 10^5 OBs, where 98 per cent of the larvae were harvested. Even though the 90 per cent harvested treatment group yielded maximum OBs per larva, it could

TABLE 2. Max. mean OB yield per larva and max. mean OB yield per unit diet against different doses for different stages

Larval instar	Mean max. OB yield per larva	Percentage larval harvest	Mean max. OB yield unit diet	Log. dose	Mean larval wt. at dosing	Age at dosing	Incubation period (days)	Mean larval wt. (mg) at harvest
III	1.67×10^8	90	1.4×10^8	2	11	4	72	36
IV	3.6×10^9	90	3.2×10^9	6	31.5	6	72	72
	3.4×10^9	98	3.3×10^9	5	31.5	6	72	76
V	3.4×10^9	100	2.3×10^9	6	95	8	84	176

TABLE 3. Regression models for individual larval stages for predicting OB yield per unit diet

Instar	Regression model	Adjusted R^2	F	P
III	Log. OB yield per unit diet = 11.981 (1.305) + 2.027 (0.527) (log. (dose × incubation period)) − 2.112 (0.531) (log. dose) − 0.038 (0.015) (incubation period)	37.7	13.699	>0.0001
IV	Log. OB yield per unit diet = 5.95 (0.832) + 4.02 (0.294) (log. (dose × incubation period)) − 3.84 (0.295) (log. dose − 0.0624 (0.006) (incubation period)	77.3	113.425	>0.0001
V	Log. OB yield per unit diet = 9.38 (0.896) + 2.579 (0.316) (log. (dose × incubation period)) − 2.436 (0.317) (log. dose) − 0.0306 (0.006) (incubation period)	67.3	68.995	>0.0001

yield only 3.2×10^9 OBs per unit diet, which was at an expense of 10^6OBs. Here, the lower mean yield per larva was compensated by 98 per cent harvest rate.

Prediction model for OB yield per unit diet

To enable the prediction of OB yield per unit diet, the functional relationship between the same and the larval stage, inoculum dose and incubation period was brought out by multiple linear regression analysis. The prediction models for OB yield per unit diet for different stages are presented in Table 3.

Based on the model summary obtained for predicting OB yield per unit diet for different stages, it was found that the major influences on OB yield were the interaction between dose and incubation period followed by the dose and the incubation period. On evaluating the model adequacy, it was found that the adjusted R^2 value equals 37.7 per cent for third instar, 77.3 per cent for fourth instar and 67.3 per cent for fifth instar.

TABLE 4. Mean maximum OB yield per unit diet obtained at different temperatures

Dosage OBs per larva	Temp (°C)	No. of larvae tested	Mean max. OB yield per unit diet
	15	43	$3.68 \times 10^{8}{}_{b}$
10^4	25	38	$7.82 \times 10^{8}{}_{a}$
	35	39	$1.83 \times 10^{8}{}_{c}$
	15	36	$1.41 \times 10^{9}{}_{e}$
10^5	25	38	$3 \times 10^{9}{}_{d}$
	35	42	$7.05 \times 10^{8}{}_{f}$
	15	34	$4.35 \times 10^{8}{}_{h}$
10^6	25	46	$9.25 \times 10^{8}{}_{g}$
	35	45	$2.17 \times 10^{8}{}_{i}$

In a column, means followed by the same subscript do not differ significantly by LSD ($P = 0.05$)

From the R^2 values it is evident that the prediction reliability of the model was more for fourth instar than the third and fifth instar larvae.

Optimum temperature regime

The maximum OB yield obtained per unit diet for fourth instar larvae through different doses and different temperatures are presented in Table 4. In the three doses tested the maximum OB yield per unit diet was obtained from the larvae incubated at 25 °C (3×10^9 OBs at 10^5 OBs/larva) followed by 15 °C (9.25×10^8 at 10^6 OBs/larva) and 35 °C (7.82×10^8 at 10^4 POBs/larva).

It is evident that the temperature is one of the factors determining the productivity of the virus. Of the three doses and three temperature regimes tested, the maximum OB yield per unit diet was registered at the dosage 10^5 OBs per larva and the temperature 25 ± 2 °C. The virus yield at this temperature-dosage combination was significantly higher than that in the rest of the combinations. In the case of all the other dosages tested, maximum virus yield was obtained at 25 ± 2 °C followed by 15 ± 2 °C and 35 ± 2 °C.

DISCUSSION

A central problem associated with *in vivo* mass production is that of obtaining maximum virus production at the expense of minimal requirements. From this study,

it is evident that the larval stage, inoculum dose, incubation period, and temperature exerted a great control over NPV yield as well as percentage of harvestable larvae.

When the data on the virus production for different larval stages were analysed, it was found that the mean maximum OB yield per unit diet for fourth and fifth instar larvae was registered against a dose of 10^5 and 10^6 OBs per larva respectively. However, in the case of third instar larvae the same was against a dose of 10^2 OBs per larva. Greater susceptibility of younger larvae might be expected to lead to lower yields associated with the reduced killing time. For any given dose, survival of infected larvae was found to drop with increase in the incubation period and with increased dose. Destruction of midgut epithelial cells was proposed to be the reason for severe impairment of growth of *Trichoplusia ni* at high doses of AcMNPV (van Beek *et al.*, 1990).

For third instar *H. puera* larvae, it was found that manipulating the inoculum dose had only a limited effect on productivity. By increasing the dose beyond 10^2 per unit diet could not increase the NPV production further. For fourth instar larvae by increasing the dose from 10^4 to 10^5 raised NPV production significantly, but increase in the dose beyond 10^5 could not increase virus yield. The rise in mean yield found between 10^4 and 10^5 could be due to the increased proportion of the inoculated larvae getting successfully infected rather than increase in the OB per unit diet. At the lower doses, less number of larvae became infected successfully while at the optimum dose, all were infected and showed elevated OB counts. For fifth instar larvae this optimum dose might be 10^6 OBs per unit diet. Even though it may be possible to increase the yield by increasing the dose further, it would be at the expense of larvae inept to harvest. It seems that for larvae of a given age, there is a threshold dose of NPV needed to overcome host resistance and initiate a successful infection. In the case of fourth and fifth instar larvae inoculated with lower doses (10^2 or 10^3 OBs), a high proportion of the larvae appeared to resist infection to the initial site of entry, the midgut epithelia. The mechanism of resistance might be rapid sloughing of the midgut epithelium at the site of entry (Engelhard and Volkman, 1995; Grzywacz *et al.*, 1998; Washburn *et al.*, 2003).

On comparing the weight increase at the time of harvest to the weight at dosing with respect to the three larval stages, it was evident that when the fifth instar larva attains only one half of the initial weight third instar attains thrice the initial weight and fourth instar larva attains almost two half of the initial weight (Table 2). Like-wise the productivity per unit diet was found to be more for the fourth instar larvae than the third and fifth instar larvae. This differential behavior of three larval instars can be attributed to the maturation response met within as larva ages (Biji, 2004). It has been suggested that the enzyme ecdysteroid UDP-glucosyl transferase encoded by the *egt* gene probably serves to maximize virus production through prolonging the feeding period of the host which is a purposeful strategy of the virus to maximize its growth period/survival advantages (Miller, 1994).

The time of harvesting is found to play a major role in determining virus productivity. Maximum productivity was obtained when the infected larvae were

harvested between 60 and 96 h p.i. which is found to be dose dependent. In *Spodoptera littoralis*, the peak virus productivity was found in insects harvested between 6 and 8 days post inoculation and before that very few OBs were found (Grzywacz *et al.*, 1998). In HpNPV infected *H. puera* larvae, the inoculum build up during the initial 12 h p.i. was at faster rate. In the case of *S. littoralis* the low OB production period corresponds to the primary infection in the midgut epithelial cells and most of the OB production occurs only later during the second phase of infection when the fat body, hemocoel and tracheal cells get infected. However, in the case of *H.puera* the fat body, hemocytes etc., get infected at first resulting in faster build up of virus inoculum during the initial phase (Biji, 2004).

The better performance of the temperature regime 25 ± 2 °C over different inoculum doses tested revealed that this regime is optimal for the multiplication of HpNPV in *H. puera* larva. At 35 ± 2 °C the OB yield per unit diet was the least. This might be due to the combined effect of reduced larval growth and the larval death at a faster rate without rendering enough time for the virus to get multiplied. At very low temperature (15 ± 2 °C) the elongated life span appears to counteract the reduction in larval growth resulting in improved OB yield per unit diet than in larvae kept at 35 ± 2 °C. Partial starvation was also met with in the larvae maintained at lower and higher temperatures due to desiccation of semi synthetic diet. Synchronization of larval growth during incubation of viral diseases can be very sensitive to temperature fluctuations (Hunter-Fujita *et al.*, 1998). For *H. zea*, it is reported that a deviation of just over 1.0 °C could result in as much as 50 per cent (larval size) deviation in a seven-day incubation period (Shieh, 1989). In *Anticarsia gemmatalis* variation in susceptibility to NPV maintained at different temperature regimes has been reported (Boucias *et al.*, 1980). In the case of the larvae maintained at 26.7 °C, the NPV replicated efficiently but the replication was slower at 15.6 °C.

To conclude, in a mass production approach, even the slightest increase in the OB yield could make the pest management strategy more economically feasible. Hence, infecting fourth instar larvae with a dose of 10^5 OBs per larva and harvesting them after an incubation period of 72 h at a temperature of 25 ± 2 °C would be ideal for getting the maximum virus yield under mass production programme. HpNPV production from *H. puera* larva, following this optimized production protocol was found to be very efficient with a 33,000-fold increase over inoculated dose. Obtained virus yield is 6.87 times more than the earlier production record of this NPV in *H. puera* larvae. However, in a commercial mass production perspective, the rearing and processing of larvae in large numbers will be a tedious process and hence studies have to be focused on automation of production steps. Apart from this, a well defined quality control system pertaining to genetic stability, virulence, microbial load, etc. is a necessity to maximize product performance, ensure product safety and reduce risks of supply failure thereby building user confidence.

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Efficacy of *Bacillus sphaericus* against *Aedes (Stegomyia) aegypti, Mansonia indiana* (Edward) and *Culex vishnui* under laboratory and field conditions

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ABSTRACT: *Bacillus sphaericus* was isolated from the gut of dead *Aedes (Stegomyia) aegypti* larva collected from submerged rice fields at Burdwan, West Bengal, India. The bacterium formed crystals and was sensitive to chloramphenicol, kanamycin, erythromycin, lomefloxacin, tobramycin, gatifloxacin, amikacin, gentamicin, sparfloxacin, amoxycillin and levofloxacin but resistant to tetracycline, ampicillin, penicillin G, ciprofloxacin, nalidixic acid, cefuroxime, cefadroxil, ofloxacin, doxycycline and norfloxacin. In the laboratory, 4.2×10^6 bacteria/ml of *Bacillus sphaericus* (Bs) killed 97.03% and 69.64% larvae of *Mansonia indiana* (Edward) and *Aedes aegypti* (Linn.), respectively, larvae within 9h, and 100% larvae of *Culex vishnui* (Theobald) in 6 h. In natural habitats, more than 90% larvae of *M. indiana* and *Cx. vishnui* and 65% of *Ae. aegypti* were infected after 12 h of application of *B. sphaericus* and no further growth of mosquito was recorded up to seven days. SDS-PAGE of crystal protein revealed that the bacteria contain 42 kDa mosquitocidal protein.

KEYWORDS: *Bacillus sphaericus*, *Aedes (Stegomyia) aegypti*, mosquito, virulence

INTRODUCTION

Mosquitoes are important vectors of numerous diseases like dengue, lymphatic filariasis, malaria, encephalitis, etc. In India and other parts of Southern Asia, *Brugia malayi* is generally transmitted by *Mansonia indiana* (Edward). *Aedes aegypti* (Linn.) is the vector of dengue and *Culex vishnui* (Theobald) is the vector of Japanese encephalitis (Cook and Zumla, 2003). *Mansonia* breeds in swamps and marshes with floating water lettuce and water hyacinth (*Eichornia* spp.). The egg masses of

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Mansonia are deposited under floating leaves of certain aquatic plants. *Mansonioides* populations thrive in the coconut retting pits which are rich in organic content and serves as food of the larvae (Cook and Zumla, 2003). While insecticides are the choice for mosquito control, its indiscriminate use has resulted in resistance development along with several health and environmental problems. Thus, non-hazardous biocontrol methods would be the best choice as safer mosquito control strategy. Several bacterial, fungal and protozoan pathogens are known as effective biocontrol agents of mosquitoes (Beier and Craig, 1985; Copping and Menn, 2001; Munsterman, 1990; Teng *et al.*, 2005) but they have not been exploited significantly (especially in India) probably due to absence of assured source of the commercial products. Nevertheless, limited research has been done to identify and exploit the natural indigenous microbial pathogens of mosquitoes in different habitats and regions of the country. In Eastern India, rice is grown mainly under flooded conditions where several mosquitoes breed and it is the exclusive habitat of *Cx. vishuni* group which is the vector of Japanese encephalitis. Therefore, information on the natural microbial pathogens of mosquitoes of the rice fields would be very useful for their control. The potent indigenous pathogens would be best suited to local conditions for biological control of these vectors. Bacteria, especially *Bacillus thuringiensis* (Bt) and *B. sphaericus* (Bs), are most potent and successful pathogens of insects including mosquitoes (Porter *et al.*, 1993; Krattiger, 1997). Both the pathogens produce inclusion bodies i.e. toxins, although susceptibility differs with mosquito species. During this investigation, a Bs was isolated from dead *Ae. aegypti* larva found in the submerged rice fields at Burdwan, West Bengal, India that effected high natural larval mortality. Although Bs is a known pathogen of mosquito, it was not reported to date from mosquitoes of Eastern India. The larvicidal factor (crystal toxin) of Bs is unique which consists of two proteins of 51 and 42 kDa (Baumann *et al.*, 1987, 1988; Porter *et al.*, 1993) both of which are required for toxicity. Therefore, the present study has been designed to isolate and characterize the pathogenic *B. sphaericus* (Bs) from *Ae. aegypti* (Linn.) larvae of the rice fields, check the crystal protein component and test virulence in the laboratory and natural habitat for biological suppression of different mosquitoes.

MATERIALS AND METHODS

Dead larvae of *Aedes (Stegomyia) aegypti* were collected from the submerged rice fields. Each larva was washed with sterile distilled water, surface sterilized with 70% alcohol followed by sterile distilled water (three times). The gut was dissected out under the laminar air flow, the gut content was aspirated through a sterile syringe, diluted up to 10^{-3} level, 100 μl of it was mixed with 100 ml nutrient agar (NA) (g/l: peptone 5, beef extract 3, agar 3, pH 7) and plated. The petriplates were incubated at 31 $\pm$ 0.1 °C in the BOD incubator for 24 h, the colonies were checked under a phasecontrast microscope and those having spherical spores and crystals were picked up, purified by dilution plating on NA plates and maintained at 4$\pm$0.1 °C on NA slants. Morphological, physiological and biochemical characters of the bacteria were studied

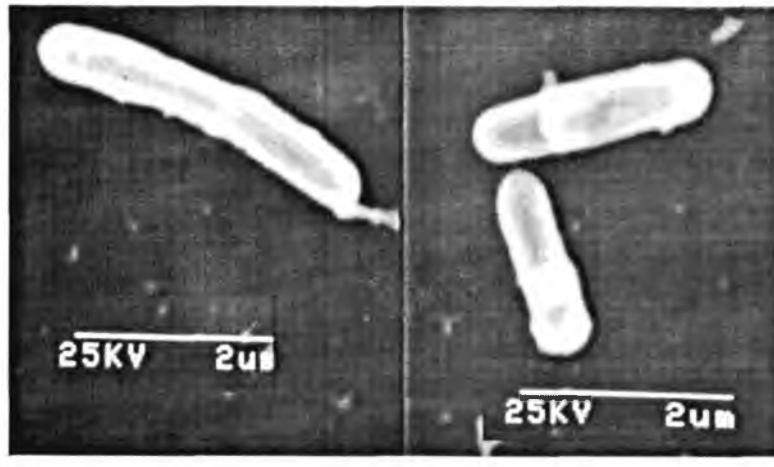

FIGURE 1. Scanning electron microscopic photograph of vegetative cells of *B. sphaericus*

following standard methods (Pelczar *et al.*, 1957; Sneath, 1986; Collee and Miles, 1989; Lacey, 1997). The bacteria were identified phenotypically following Sneath (1986). Antimicrobial test was done with standard antibiotics discs following Bauer *et al.* (1996).

The bacteria was grown on 100 ml NB (NA without agar), centrifuged at 10000 g for 10 min at 4±0.1 °C and the pellet (spore and crystal) was taken. It was resuspended in 2–3 ml sterile water, mixed with equal volume of alkaline solubilization buffer (50 mM sodium carbonate containing 10 mM dithiothreitol (DTT), pH 10) and incubated at 37± 0.1 °C for 12 h on a shaker at 25 rpm. The pH of the solubilized crystal was adjusted to neutrality with 0.5M HCl and then treated with 1/10th volume trypsin (200 U/g potency, dissolved in sterile water @ 1 mg/ml) and incubated for 3-4 h at 37±0.1 °C. An equal amount of trypsin was added and the mixture was incubated overnight at 37±0.1 °C (Attathom *et al.*, 1995), centrifuged at 8000 g for 10 min at 4±0.1 °C and the supernatant was taken. Protein concentration was determined at 280 nm through a UV-vis spectrophotometer. The trypsinized crystal protein preparation was refrigerated at 4±0.1 °C until use. Protein profile of trypsinized crystal protein was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) resolved at 5V/cm up to stacking gel followed by 15V/cm for the resolving gel (Janssen, 1994).

Bacterial smear was prepared on a cover glass, heat fixed over a flame for 1–2 sec followed by 2.5% glutaraldehyde (aqueous) for 45 min. The slides were then dehydrated passing through 50%, 70%, 90% and finally with absolute alcohol for 5 min each. The specimens were gold coated and observed under a SEM.

To determine the toxicity of *B. sphaericus* against different mosquito species, the bioassay tests were carried out at 30±2 °C; using 100 larvae (late third instar) kept in 1000 ml water in glass bowls. The larvae were exposed to the dose of 5 ml

TABLE 1. Phenotypic characterization of the bacteria (B-1) isolated from *Aedes aegypti*

Character	Observation	Character	Observation
Colony character	Circular, off white, flat, entire	Urease production	+
Bacterium (l × w, μm)	Rods with rounded ends, motile, Gram (+)ve, 2-4 × 1	Oxidase test	+
Spore(Dia., μm)	Round (0.93-1.05)	H_2S production	-
Crystal (Dia., μm)	Spherical (0.58-0.70)	Citrate test	No growth
NaCl tolerance (%)	3	Gelatinase	+
Acid and gas production	Negative	Casein hydrolysis	+
Catalase	+	Amylase	-
Indole production	-	Lipase	-
Methyl red test	-		
Vogues-Proskauer test	-	Nitrate reduction test	-
Antibiotic resistant (μg/disc)	Tetracycline (30); ampicillin (10); penicillin G (10); ciprofloxacin (5); nalidixic acid (30); cefuroxime (30); ofloxacin (5); cefadroxil (30); doxycycline (30); norfloxacin (10)	Antibiotic sensitive (μg/disc)	Chloramphenicol (30); kanamycin (30); erythromycin (15); lomefloxacin (10); tobramycin (10); gatifloxacin (10); amikacin (30); gentamicin (10); sparfloxacin (10); amoxycillin (30); levofloxacin (5)

Identity of the organism: *Bacillus sphaericus*

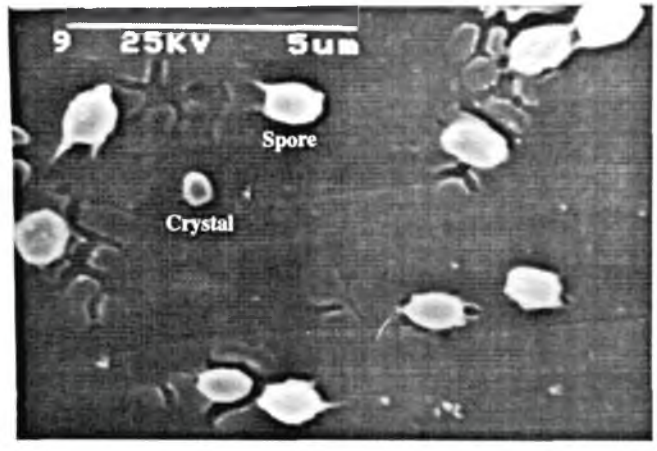

FIGURE 2. Scanning electron microscopic photograph of spores and crystals of *B. sphericus*

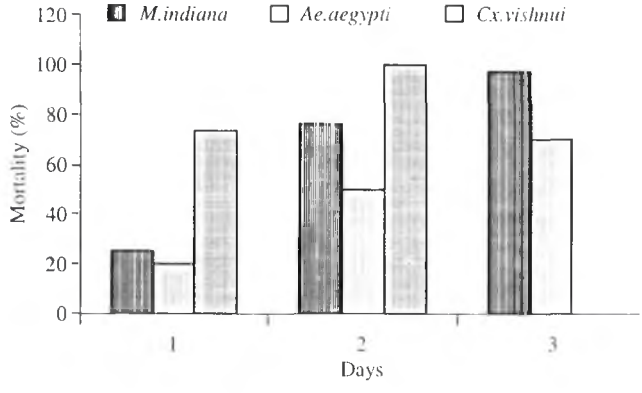

FIGURE 3. Mortality of *M. indiana*, *Ae. aegypti* and *Cx. vishnui* in the laboratory treated with *B. sphaericus*

of *B. sphaericus* suspension ($4.2x10^6$bacteria/ml) per liter of water. Each test was replicated three times at three different times along with a control and the mortality (%) was corrected following Abbott's formula.

Twelve breeding habitats (four submerged fields, four drains and four ponds) with very high larval densities were selected for the field study. Out of them, two of each breeding habitat were left untreated and remainder two were treated with *B. sphaericus* (Bs) suspension. Mosquito larvae were sampled from both treated and untreated sites by the dipper method after 24 h for one month starting from 1st June to 30th June to determine survival of the larvae. Every site was sampled using 20 dips in a spot having aggregation of large number of larvae. The contents of the dipper were transferred to a white enamel pan (15 × 30 × 4 cm) and the larvae were counted.

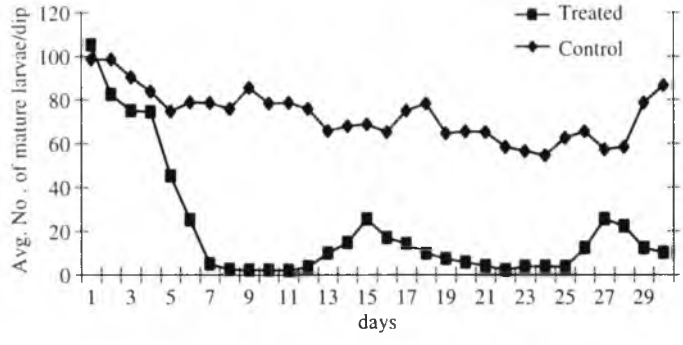

FIGURE 4. Population of mosquito larvae in treated and untreated sites on different days

RESULTS AND DISCUSSION

The bacteria formed circular, white, flat and undulate colonies (Table 1). The bacteria were Gram positive, spherical spore and amorphous spherical crystals forming aerobic rods measuring 2–4 × 1 μm (Figs. 1, 2) and did not produce acid and gas from different carbon sources (Table 1). The organisms were positive for catalase, urease, protease and oxidase but negative for H_2S production, indole production, nitrate reduction, Vogues-Proskauer test and lipase (Table 1). The organisms were sensitive to recommended doses of chloramphenicol, kanamycin, erythromycin, lomefloxacin, tobramycin, gatifloxacin, amikacin, gentamicin, sparfloxacin, levofloxacin amoxycillin but resistant to penicillin G, tetracycline, ampicillin, norfloxacin, nalidixic acid and cefuroxime (Table 1). The morphological, physiological and biochemical characters identified the isolates as *B. sphaericus* (Sneath, 1986). SDS-PAGE of crystal protein revealed that the bacteria contained 42 kDa protein. In the laboratory, application of 5 ml/l (4.2×10^6bacteria/ml) Bs suspension resulted in 97.03% and 69.04% death of *M. indiana* and *Ae. aegypti* larvae, respectively but 100% mortality of *Cx. vishnui* larvae within 6 h (Fig. 3). The observation revealed that the Bs was more effective against *Cx. vishnui* and *M. indiana* than *Ae. aegypti* larvae (Fig. 3). *Cx. vishnui* larvae always move vertically within the water. So, *Cx. vishnui* larvae may ingest more Bs (aerobic) than other larvae and may show higher mortality than *M. indiana* and *Ae. aegypti*. The results corroborate that Bs is an intestinal toxicant and potent pathogen of mosquitoes (Porter *et al.*, 1993; Krattiger, 1997; Copping and Menn, 2001, Teng *et al.*, 2005). The crystals of the organism produced a fraction of protein of about 42 kDa size. The protein is an established mosquitocidal toxin (Baumann *et al.*, 1987, 1988; Porter *et al.*, 1993), which would be effective against mosquitoes of our study also. However, the 51 kDa equivalent fraction of the toxin needs to be worked out. The average larval density per dip in the experimental site was 105.23 (Fig. 4). After the bacterial treatment, the density drastically reduced to 4.8 after 7d and 1.9 on 11th day. Then the larval population increased again and the per dip density became 25.4 on 15th day when 2nd treatment was done. The average larval density was again reduced up to 22nd day (2.1

per dip) having a lag phase up to 25th day (3.5 per dip) (Fig. 4). The third treatment was done on 27th day when average larval density per dip was 25.4 and the larval density again declined up to 30th day (Fig. 4). In the control site the average per dip density ranged from 98.6 to 54.4 in the experimental period. Therefore, this bacterial isolate was adjudged as a potential bio-control agent of mosquito larvae. The results showed that the Bs would be helpful in public health programmes for control of vector borne diseases through control of vector mosquitoes.

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Description of a new species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) from central India

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ABSTRACT: A new species, *Trichogramma latipennis* sp. n., is described and illustrated. Diagnostic characters of the genus *Trichogramma* Westwood are given in brief. The new species *T. latipennis* is closely related to *T. brevicapillum* Pinto & Platner in having its antennae with flagellum short and stout, about four times as long as wide; having small and thick hairs with blunt tips. But the former can easily be distinguished from the latter by having male genitalia with the position of chelate structures which are far behind the tips of gonoforeceps, longest flagellar seta about one and a half times as long as maximum flagellar width, and ovipositor about as long as hind tibia.

KEYWORDS: Hymenoptera, Trichogrammatidae, *Trichogramma*, *latipennis*, *brevicapillum*

INTRODUCTION

The concept of Integrated Pest Management is widely accepted now and biological control is one of its vital components. *Trichogramma* species have been utilized successfully all over the world for controlling several serious lepidopterous pests of agricultural and forestry importance (Debach and Rosen, 1991). Trichogrammatids are very minute, 0.2–1.0 mm long, widely distributed, and attack the eggs of insects mostly of Lepidoptera and Hemiptera. New species of *Trichogramma* recorded recently from India include *T. flandersi, T. chilotraeae, T. achaeae* (Nagaraja and Nagarkatti, 1969), *T. hesperidis, T. agriae, T. pallidiventris, T. plasseyensis, T. poliae, T. raoi* (Nagaraja, 1973), and *T. brevifringiata* (Yousuf and Shafee, 1987). One new species, *Trichogramma latipennis* sp. n., from central India is described in this paper.

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Genus *Trichogramma* Westwood (1833)

Trichogramma Westwood, 1833: 444.

Type species: *Trichogramma evanescens* Westwood, by monotypy.

Diagnosis

Members of the genus *Trichogramma* are easily recognized by having their female antennae with 2-segmented funicle and one segmented club; fore wings with sigmoid venation, discal setae arranged in rows; vein track RSI present. Male genitalia with broad phallobase, digiti and claspers well developed (Doutt and Viggiani, 1968; Yousuf and Shafee, 1987).

The genus *Trichogramma* Westwood is known to contain 143 species (Lin, 1994; Yousuf *et al.*, 2004), including one new species; of which 14 species have been recorded from India.

The new species *T. latipennis* is closely related to *T. brevicapillum* Pinto & Platner, from which it can easily be separated by the following key characters.

1. Male antennae with longest flagellar seta 1.17 times as long as maximum flagellar width, female antenna with club more than two times as long as wide; male genitalia with chelate structures a little behind the tips of gonoforeceps, Ovipositor shorter than hind tibia ..... *T. brevicapillum* Pinto & Platner
- Male antennae with longest flagellar seta about 1.5 times as long as maximum flagellar width, female antenna with club less than two times as long as wide; male genitalia with chelate structures far behind the tips of gonoforeceps; Ovipositor about as long as hind tibia ..... *T. latipennis sp. n.*

Among Indian species, *T. latipennis* is closely related to *T. plasseyensis* Nagaraja by having its chelate structures far behind the tips of gonoforceps and ovipositor as long as hind tibia but the former can easily be distinguished from the latter by having antennae with flagellum short and stout, about four times as long as wide; having small and thick hairs with blunt tips, longest flagellar seta about one and a half times as long as maximum flagellar width.

Trichogramma latipennis sp. n. (Fig. 1)

Male

Head brown-yellow, slightly wider than long in facial view; ocelli orange, arranged in obtuse triangle; eyes red. Antennae (Fig. 1A) brownish-yellow; scape cylindrical, slightly less than four times as long was wide; pedicel about two times as long as wide; one ring segment present; flagellum short and stout, slightly more than four times as long as wide; 4 I short, stout and blunt, flagellar setae (Fig. 1B), longest of which about one and a half times as long as maximum width of flagellum.

Thorax brownish yellow with fuscus sides of pronotum, mesonotum and pleurae. Fore wings (Fig. 1C) hyaline, except the area beneath venation lightly infuscated, about two times as long as wide; disc densely setose, discal setae arranged in rows;

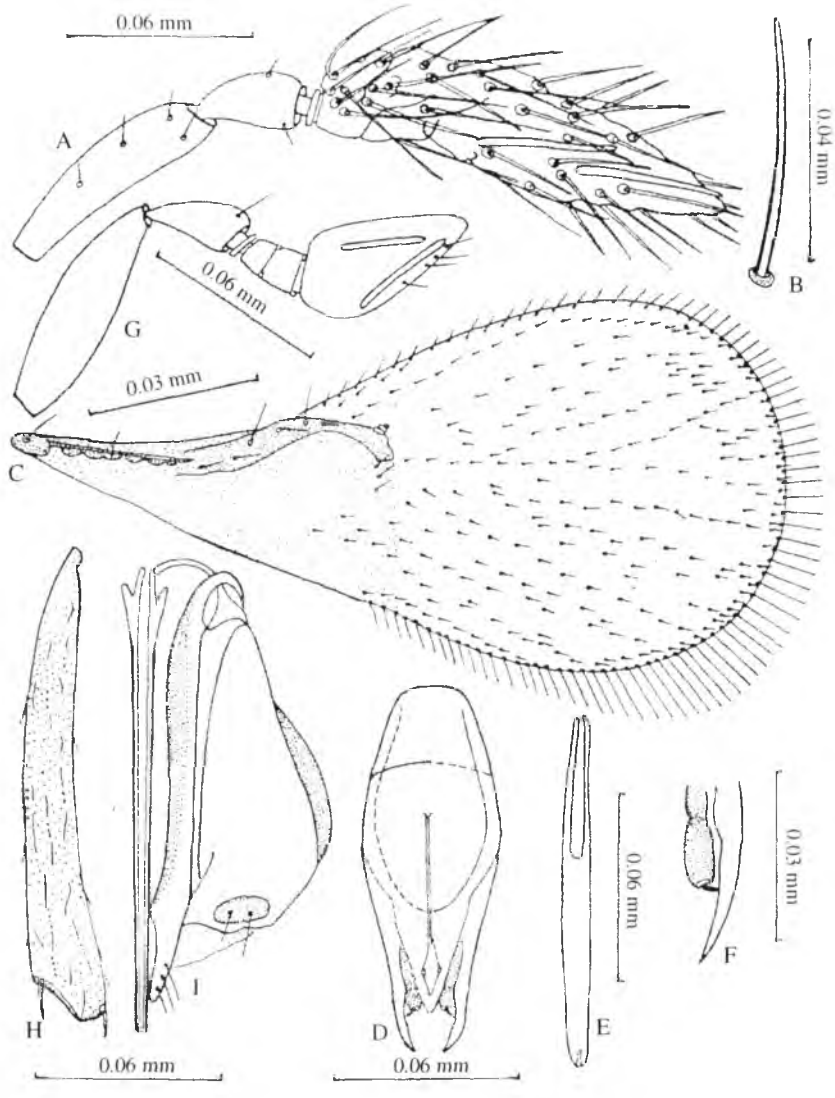

FIGURE 1. *Trichogramma latipennis* sp. n.: A. antenna ♂; B. Flagellar seta ♂; C. fore wing ♂; D. genitalia ♂; E. aedeagus ♂; F. Chelate & gonoforceps ♂; G. antenna ♀; H. hind tibia ♀; I. genitalia ♀.

costal cell narrow; Vein track RS1 with three setae; RS2 with 6 setae; r-m with 18 setae; between RS2 & r-m 32 setae; marginal fringe long, about one fifth the wing width. Legs yellow with coxae lightly infuscated.

Abdomen yellow, slightly longer than thorax; genitalia with DEG moderately narrow, (Fig. 1D), CS far below the level of GF (Fig. 1F). Aedeagus (Fig. 1E) longer

than apodemes, together slightly shorter than entire genitalia and also shorter than hind tibia (Fig. 1H). Body length 0.42 mm.

Female

Body colour same as male. Antennae yellow; scape slightly more than three times as long as wide; pedicel about two times as long as wide; single ring segment present; funicle two-segmented, both segments combined about one and a half times as long as wide; club solid; less than two times as long as wide. Ovipositor hidden, tip of ovipositor slightly exserted; ovipositor about as long as hind tibia.

Body length : 0.43 mm

Holotype ♂, allotype ♀, INDIA : Madhya Pradesh; Chhindwara, Silewani Ghati, 09.1.2006; by sweeping method, M. Yousuf.

Holotype and allotype have been deposited in the Insect Museum, Tropical Forest Research Institute, Jabalpur, M. P., India.

Comments: The new species *T. latipennis* is closely related to *T. brevicapillum* Pinto & Platner by having its male antennae with flagellum short and stout, about four times as long as wide; having small and thick hairs with blunt tips, but the former can easily be distinguished from the latter by having male genitalia with chelate structures far behind the tips of gonoforeceps, longest flagellar seta about one and a half times as long as maximum flagellar width; female antennae with club less than two times as long as wide; ovipositor as long as hind tibia.

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Influence of temperature on biological parameters of *Goniozus nephantidis* Muesebeck, a promising parasitoid of the coconut black headed caterpillar *Opisina arenosella* Walker

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ABSTRACT: The influence of temperature on the biological attributes of the parasitoid *Goniozus nephantidis* (Muesebeck) was studied at various temperature regimes and RH in the laboratory. The fecundity, parasitizing efficiency, percent adult emergence and adult longevity were significantly higher when reared at 32 and 34 °C than when reared at 24, 26 and 36 °C. Temperature had no significant effect on the sex ratio. Temperatures of 32–34°C at 65% RH would be ideal for mass multiplication of the parasitoid under *in vitro* conditions.

KEYWORDS: biological parameters, *Goniozus nephantidis*, temperature

The black-headed caterpillar Opisina arenosella Walker is one of the serious pests of coconut palms and attempts to manage the pest are mostly by inundative releases of the parasitoids (Desai *et al.*, 2003). Among the parasitoids, *Goniozus nephantidis* Musebeck is being widely used in the biological control programmes. Varying extent of field parasitisation has been reported from different states, viz, 47.6% from Kollam district of Kerala (Sathiamma *et al.*, 1996), 31% from Mahua district of Gujarat (Kapadia and Mittal, 1993), 57.6% from Thane district of Maharashtra and 48% from Bangalore district of Karnataka (Nadarajan and Channabasavanna, 1980).

Effective biological control of the pest is achieved when large scale release of the parasitoids is made, which in turn is dependant on mass production on a commercial scale. Scale up of production in the laboratories is governed by the inherent biological attributes of the parasitoid, the predisposing abiotic factors, temperature and relative humidity. Temperature was reported as critical factor in determining the survival and development of immature stages and the reproductive performance of the parasitoids

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(Chandrika Mohan *et al.*, 2004). No oviposition was reported to occur at 14 and 37 °C (Remadevi *et al.*, 1978), while 30 °C and 70% RH was conducive for maximum hosts parasitised, fecundity and adult longevity (Dharmaraju and Pradhan, 1977; Hardy *et al.*, 1992). In the present studies the influence of temperature on the biological attributes of the parasitoid was studied with a view to improving the existing mass culture techniques.

The investigation was made in the laboratory at the Project Directorate of Biological Control, Bangalore, Karnataka, India .The stock culture of the parasitoid was obtained from field-collected cocoons and parasitized host larvae of *O. arenosella*. Ten newly emerged adults of the parasitoid in the ratio 1:2 males to females were released into plastic containers (5 cm × 7 cm) covered with muslin cloth. The adults were fed with undiluted honey provided as droplets on an alkathene paper strip. After a pre-oviposition period of 4–5 d, the females were separated and provided with suitable larvae of the rice moth *Corcyra cephalonica,* obtained from a culture maintained in the laboratory.

A single larva of C. *cephalonica* was offered to the parasitoid in each vial. After parasitisation (24–36 h exposure), the paralyzed larvae containing eggs were removed, placed on tissue paper and kept in a plastic jar (10 cm × 5 cm) for cocoon formation and adult emergence. The rearing was done at temperatures of 24, 26, 32, 34 and 36 °C.

The fecundity, percentage larval parasitisation, percentage pupation, adult emergence, adult longevity and sex ratio were recorded and statistically scrutinized by ANOVA. Eight generations with three replications under each temperature was studied.

The data are presented in Table 1. The fecundity (54.62 and 58.2 eggs per female), parasitizing efficiency (8.54 and 9.21 larvae per female), percent adult emergence (85.8 and 86.41%) and adult longevity (54.61 and 58.61 days) were significantly higher and on par when reared at 32 and 34 °C. Dharmaraju and Pradhan (1977), Hardy *et al.* (1992) and Venkatesan and Jalali (2007) had reported that 30 °C and 70% RH resulted in higher fecundity and adult life span. Chandrika Mohan *et al.* (2004) found that a temperature range of 30–35 °C increased fecundity, parasitisation and longevity than lower temperature regime of 20–25/28 °C

Increased temperatures reduced the duration of development (9.92 d at 36 °C compared to 15.62 d at 24 °C). However, significant decrease in fecundity (50.24 eggs/female), parasitising efficiency (8.02 larvae/female), pupation (82.22%) and adult emergence (78.42%) were recorded at 36 °C and 24 °C. Remadevi *et al.* (1978) recorded no oviposition at temperatures of 14 °C and 37 °C and maximum eggs were laid at 29–33 °C. Hekal (1990) reported that duration of immature stages of the parasitoid decreased with increase in temperature up to 35 °C from 25 °C. Murthy *et al.* (2006) could not find adverse changes in the biological parameters of the parasitoid reared under fluctuating temperature regime of 26 to 32–36 °C in a day–night cycle.

The sex ratio was female biased, irrespective of the temperature regime. No significant difference was observed under the various temperatures. Higher temperatures did

TABLE 1. Biological attributes of *Goniozus nephantidis* reared at various temperatures

Biological Parameter	Temperature (°C)					CD $P>0.05\%$
	24±1	26±1	32±1	34±1	36±1	
Fecundity (No. of eggs/♀)	36.42±0.62	49.64±0.86	54.62±0.75	58.20±0.82	50.24±0.86	2.12**
Parasitising efficiency (%) (No. of larvae parasitised/♀)	4.42±0.80	6.83±0.80	8.54±0.73	9.21±0.84	8.02±0.82	2.27**
Percentage pupation (Cocoons formed)	71.64±0.62	78.77±0.77	86.50±0.62	88.60±0.64	80.22±0.66	4.47**
Adult emergence (%)	64.60±0.66	72.41±0.63	85.80±0.74	86.41±0.86	74.22±0.79	5.82**
Adult longevity (days)	43.62±1.80	49.52±2.7	54.61±2.92	58.61±2.84	52.20±2.24	4.12**
Sex ratio (♂:♀)	1:1.8	1:2.22	1:2.55	1:2.82	1:2.14	NS
Total Development period (days)	15.62	13.78	11.72	11.54	9.63	2.12*

not impair the duration of the developmental activities nor impair the reproductive capability. Kapadia and Mittal (1986) observed a sex ratio of 1:1.31 when the parasitoid was reared at ambient room temperatures.

Higher temperature tolerance observed in the present study is a desirable attribute in summer months of April–May (Gubbaiah and Revanna, 1989; Sathiamma *et al.*, 1996; Mohanty *et al.*, 2000). *In vitro* production of the parasitoids for field releases can be ideally done at 32–34 °C than at ambient room temperatures. Field releases of the parasitoid would be more effective when the prevalent temperatures are between 32–35 °C coinciding with the peak pest activity.

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Seasonal incidence of sugarcane woolly aphid, *Ceratovacuna lanigera* Zhnt. (Hemiptera: Aphididae) in Assam, India

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ABSTRACT: The seasonal incidence of sugarcane woolly aphid in Assam was studied by population assessment at two locations (Jorhat and Golaghat) in Assam. The population appeared in early rainy season (April/May) and then gradually increased, remained high during July to November (autumn and early winter), and reached its peak in September in both locations. The population was low or absent in late winter and summer. The pest population had significant positive correlation with temperature (maximum and minimum), morning humidity and sunshine duration and significant negative correlation with relative humidity (evening) at both locations. Rainfall and wind speed showed positive correlation with population at Golaghat and negative correlation at Jorhat. Regression analysis showed that 65.9% to 55.3% of the variation in the population can be attributed to the combined effect of meteorological factors.

KEYWORDS: *Ceratovacuna lanigera*, sugarcane pest, Assam

Though the sugarcane woolly aphid, *Ceratovacuna lanigera* Zhnt. (Hemiptera: Aphididae) is already established as a major pest in countries like Taiwan and Indonesia, in India it has only recently gained major pest status. As many as 200 species of insects have been reported to damage sugarcane crop in India (Atwal and Dhaliwal, 2002) and those associated with sugarcane in Assam are mainly borers and sap suckers (Phukan, 1978). Among the sucking pests, *C. lanigera* is the most serious. The insect was first reported from West Bengal in 1958 and later from other parts of the region (Basu and Banerjee, 1958), in recent years this insect has been gradually attaining major pest status in the sugarcane growing belts of Assam as well as Maharashtra, Karnataka, Bihar and West Bengal. In Assam, the pest has been reported by Phukan (1978) and Sarma *et al.* (2007). Research on *C. lanigera* in India,

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especially in Assam, is very limited, because earlier it was considered as a minor pest of sugarcane (Ghosh, 1974).

Two field experiments were conducted in two locations, viz., Instructional-cum-Research (ICR) Farm, Assam Agricultural University (AAU), Jorhat and Sugarcane Research Station (SRS), Buralikson, Golaghat, during 2004–2005 to understand the seasonal occurrence of the pest in Assam. In each location, one plot with an area of 500 m^2 was selected for observations. The crop was maintained by following the recommended package of practices and was kept completely free from any insecticide or herbicide use.

Population of *C. lanigera* was recorded at fortnightly intervals starting from the first fortnight of April 2004 and continued upto the second fortnight of February 2005. Plant inspection method was adopted for collecting data. Forty sugarcane plants were assessed. From each infested cane three leaves (one each from top, middle and bottom portions of the cane) were selected and from each leaf the number of aphids on the surface at three different locations each covering 9 cm^2 area were counted. A window (3 cm × 3 cm) was used for the purpose. Mean temperature, relative humidity, rainfall, wind speed and bright sunshine hours were recorded at the two locations and for every fortnight. Data on population variations and weather factors were statistically correlated and the impact of independent variables on the dependent variable was assessed with multiple regression analysis.

The data in Table 1 reveals that the period of occurrence and the intensity of incidence of *C. lanigera* were higher at Jorhat than at Golaghat. Pest appeared in the field in the first fortnight of April 2004 and persisted up to the first fortnight of January at Jorhat while the corresponding period of occurrence at Golaghat was from second fortnight of May 2004 to first fortnight of January 2005. When the pest population reached the peak in second week of September the aphid counts were 46.67/9 cm^2 leaf area and 8.73/9 cm^2 area in Jorhat and Golaghat respectively. In the observations the population at Golaghat was conspicuously lower than those at Jorhat. These variations can possibly be attributed to the differences in the crop or microclimatic factors.

Regarding the influence of different seasons of the year on the incidence and intensity of pest occurrence, the data did not show conspicuous variations between the two locations. The population was very low or absent in summer season (February–April). In the rainy season (May–July) the count showed a gradual increase (from 2.63 to 21.51 and from 0 to 8.28 at Jorhat and Golaghat, respectively). In the first two months of post monsoon season (Autumn–August to October) the population build up was rapid and the peak was observed at both the places by the second fortnight of September 2004. From the last month of autumn, ie. October, the population showed a declining trend and it reached a very low/zero level by the first fortnight of January 2005. Thus the population remained conspicuously higher in autumn and early winter season (July–November), the peak being in September. The peak period of incidence was reported as September by earlier workers also (Phukan, 1978; Gupta and Goswami, 1995; Rabindra *et al.*, 2002). They also recorded the absence of the pest in the field from January to March.

TABLE 1. Seasonal incidence of *Ceratavacuna lanigera* on sugarcane at Jorhat and Golaghat, Assam

Period of observation	Mean population in 9 cm^2 leaf area at	
	Jorhat	Golaghat
2004, April I	2.63	0.00
2004, April II	6.08	0.00
May I	8.96	0.00
May II	10.13	2.26
June I	12.27	4.37
June II	12.35	6.51
July I	14.38	7.41
July II	21.51	8.28
August I	25.86	11.79
August II	35.12	16.71
September I	39.13	22.98
September II	46.67	28.73
October I	33.50	20.93
October II	28.85	16.84
November I	26.40	13.40
November II	24.57	8.65
December I	22.06	6.54
December II	17.96	3.61
2005, January I	14.31	1.60
2005, January II	5.42	0.00
February I	1.62	0.00
February II	0.00	0.00

I, First Fortnight; II, Second Fortnight

Correlation analysis of the data revealed that the aphid population had significant positive association with maximum and minimum temperatures, relative humidity (evening) and bright sunshine hours at both the locations and a significant correlation was seen with relative humidity (morning). Total rainfall and wind speed showed a positive correlation with the population at Golaghat while the correlation was negative at Jorhat. The positive influence of higher temperature on the population build up of the pest was reported earlier also (Dahms and Pianter, 1940; Hackerott and Harvey, 1959). Multiple regression analysis of the data revealed that 66.9% and 55.3% of the variations in the population of *C. lanigera* can be attributed to the combined effect of various meteorological factors.

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Influence of *Varroa jacobsoni* Oudemans parasitization on some enzymes of carbohydrate metabolism in *Apis mellifera* L. worker brood

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ABSTRACT: Total protein concentration in non-infested pupa of *Apis mellifera* L. infested by the mite parasite, *Varroa jacobsoni* was 0.26 mg/ml compared to 0.176 mg/ml in infested pupa. Specific activities of the enzymes, glucose 6-phosphatase, hexokinase, and acid and alkaline phosphatase were higher in *V jacobsoni* infested pupa compared to healthy pupa.

KEYWORDS: *Apis mellifera, Varroa jacobsoni*, glucose 6-phosphatase, hexokinase, phosphatases, mite, honey bee

The mite *Varroa jacobsoni* was first found parasitizing the Indian honey bee, *Apis cerana* (Oudemans, 1904). It extended its host range to *Apis mellifera,* parasitising worker brood as well as drone brood and has now become a serious mortality factor worldwide. Honey bee parasitization by *Varroa* is capable of causing biological, behavioural, morphological and physiological abnormalities in the bee colony. The colony infested with *V jacobsoni* can be recognized by various symptoms such as spotty brood pattern, dead larvae, pupae, and workers and drones with deformed legs, wings and shortened abdomen.

Varroa feeds on the hemolymph of adult bee, larvae and pupae. Mother mite produces wounds on the host cuticle with its saw toothed chelicerae during feeding (Martin, 1997). Ball (1997) opined that feeding by mites caused hemolymph depletion in the host and facilitated viral and bacterial infection. Chronic septic injuries to brood led to pathophysiological changes involving a variety of enzyme functions. *Varroa* immunosupresses the honey bee (Yang and Cox-Foster, 2005).The suppressed immunity is due to the reduction in the gene encoding antimicrobial peptides and immunity-related enzymes. In the present study the effect of *V. jacobsoni* parasitization on the activity of the enzymes glucose 6-phosphatase, hexokinanse, and acid and alakaline phosphatases on the worker brood of *A. mellifera* was investigated.

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TABLE 1. Effect of mite infestation on enzyme activities of *Apis mellifera* worker brood

Enzyme	Substrate concentration (ml)	Specific enzyme activity per mg protein	
		Non-Infested	Infested
Glucose 6-phosphatase	1.2	0.033 ±0.002	0.113 ±0.002
	0.6	0.025 ±0.001	0.100 ±0.002
	0.3	0.017 ±0.001	0.062 ±0.003
Hexokinase	1.2	0.031 ±0.002	0.046 ±0.001
	0.6	0.020 ±0.001	0.030 ±0.001
	0.3	0.010 ±0.001	0.015 ±0.001
Acid phosphatase	2.4	0.227 ±0.001	0.268 ±0.004
	1.2	0.163 ±0.005	0.179 ±0.001
	0.6	0.069 ±0.001	0.099 ±0.004
Alkaline phosphatase	2.4	0.236 ±0.004	0.292 ±0.001
	1.2	0.117 ±0.005	0.134 ±0.007
	0.6	0.034 ±0.001	0.055 ±0.003

± Shows Standard Deviation

The most infective stage in the life cycle of host is the late pupa (the brown eye stage) (Kumar *et al.*, 1993). A random sample of 10 infested and 10 non-infested worker pupae was taken from the colonies maintained by the Department of Zoology, Panjab University, Chandigarh. Each pupa was taken in 1 ml of saline and was homogenized electrically. The homogenate was used for further analysis. Protein was estimated by Lowry's method (Lowry *et al.*, 1951). Specific activities of glucose 6-phosphatase, hexokinase, and acid and alkaline phosphatases were estimated by following the standard protocols (Freeland and Harper, 1959; Crane and Sols, 1955).

The total protein concentration in the whole body extract of healthy pupa was found to be 0.26 mg/ml as compared to 0.176 mg/ml in the infested pupa. Activities of all the enzymes studied were higher in infested pupa than in uninfested pupae as shown in Table1.

Physiological interference due to mite infestation was reported by Ball (1997) who observed depletion in host hemolymph as a consequence of feeding by the mite. The rate of loss of hemolymph was variable depending upon the number of parent mites during bee development.

In the present investigation the activity of hexokinase was greater in infested pupa (0.046/mg protein) as compared to non-infested (0.031/mg protein). Infested bees are restless and irritated because of the presence of the mite. This leads to increased muscle activity, probably due to movement in attempts to shed off the mite adhering to the thoracic region where the mites are particularly attached. The activity of glucose 6-phospahtase also showed elevation as result of mite infestation (Table 1). Surholt and Newsholme (1981) observed strong correlation between the activities of glucose

6-phosphatase and hexokinase. It was suggested that the role of glucose 6-phosphatase in muscle is either to produce glucose from glucose–phosphate derived from glycogen or to provide the enzymatic basis for the mechanism that regulates the rate of glucose phosphorylation. The increase in activity of acid and alkaline phosphatases can be correlated to the above observation. Recently Lipinski and Zoltowaska (2005) reported significantly high activity of three anti-oxidant enzymes in *Varroa* infested drone pre-pupae and suggested that oxidative stress might be one of the pathogenic pathways of Varroasis.

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Optimum number of sprays of insecticides for controlling mango shoot gall, *Apsylla cistellata* Buckton (Hemiptera: Psyllidae)

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ABSTRACT: An experiment was conducted to find out the optimum number of sprays of commonly used insecticides, viz. dimethoate, quinalphos, endosulfan, monocrotophos, thiamethoxam and imidachloprid for control of mango shoot gall. The spraying was started from the middle of August and repeated at 15 day intervals. Dimethoate, at 2 ml/l and three sprays was found most effective for controlling the gall formation. Endosulfan was the least effective against the pest.

Mango (*Mangifera indica* L.), grown all over the country, is infested by a number of species of insects. *Apsylla cistellata* is a serious pest of mango in plains of northern India, Nepal and Bangladesh. In general, yield from the crop is largely dependent on the management of pest. Prophylactic pesticide application is still resorted to for the management of pests like gall insects though minimum use of pesticides in the agro-ecosystem is the adopted practice in modern pest control technology. Hence the pesticides commonly used for the control of *A. cistellata* in Pantnagar were tried in varying doses and number of sprayings to arrive at the minimum effective levels of dose and frequency. Six insecticides, viz. dimethoate, quinalphos, endosulfan, monocrotophos, thiamethoxam and imidachloprid were tested. There were 37 treatments arranged in asymmetrical factorial completely randomized design, each with five replications. These included two concentrations (1ml and 2 ml) of the insecticides applied once (N1) twice (N2) or thrice (N3), starting from 15 August and then at 15 days intervals. The sprays were applied on leaves bearing eggs. Observations were taken on the number of nymphs died and total number of nymphs feeding in one cm length on the leaf midrib, one week after spraying. Embryos secreting the whitish exudate were considered alive while others were counted as dead. Observations were recorded on the number of galls formed (20 cm length/ branch) and panicles emerging, one month after spraying. The study was conducted during 2006–07 and 2007–08.

The results are presented in Table 1. During both the years the highest nymphal mortality and minimum number of galls were observed on the trees which were treated

TABLE 1. Efficacy of varying doses and frequency of insecticides in controlling mango shoot gall, *Apsylla cistellata* in Pantnagar

Treatment	Mean percent nymphal mortality		Mean No. of galls		Mean No. of panicles emerged	
	2006–07	2007–08	2006–07	2007–08	2006–07	2007–08
dC_1N_1	70.50	80.00	2.25	3.25	1.25	1.20
dC_1N_2	72.75	81.00	1.75	2.25	2.20	2.25
dC_1N_3	76.25	91.00	2.00	0.00	2.00	3.25
dC_2N_1	72.50	90.75	0.50	2.25	2.75	2.25
dC_2N_2	74.00	87.75	0.00	0.50	3.50	3.00
dC_2N_3	85.00	97.75	0.00	0.00	3.75	3.25
qC_1N_1	41.25	58.75	4.25	4.25	0.75	0.25
qC_1N_2	52.25	63.75	2.50	1.50	1.25	2.25
qC_1N_3	53.25	69.75	2.00	2.50	1.50	2.00
qC_2N_1	61.25	61.75	1.25	1.25	1.75	2.50
qC_2N_2	66.50	70.75	1.00	1.00	2.25	3.25
qC_2N_3	71.50	76.75	0.75	0.75	2.50	3.50
eC_1N_1	11.00	10.75	16.00	17.50	0.05	0.05
eC_1N_2	10.50	11.75	16.50	16.75	0.05	0.07
eC_1N_3	10.00	12.25	15.50	13.75	0.75	0.40
eC_2N_1	13.00	13.25	12.75	12.75	0.25	0.08
eC_2N_2	13.75	16.50	9.75	8.50	0.50	0.25
eC_2N_3	15.00	14.25	8.50	8.25	0.75	1.00
mC_1N_1	65.25	71.75	3.25	3.00	1.25	1.25
mC_1N_2	68.50	75.75	2.50	2.50	1.50	2.25
mC_1N_3	70.00	75.75	2.50	2.25	1.75	2.00
mC_2N_1	67.00	71.50	1.00	3.50	2.25	1.25
mC_2N_2	70.25	74.50	0.50	3.25	3.25	1.50
mC_2N_3	73.25	78.75	0.25	2.25	3.50	2.25
tC_1N_1	8.50	9.75	14.50	15.50	0.50	0.70
tC_1N_2	7.25	8.50	13.50	14.50	0.50	0.25
tC_1N_3	9.00	10.25	12.50	13.75	0.25	0.00
tC_2N_1	10.75	12.50	13.25	12.25	1.00	0.25
tC_2N_2	12.50	13.50	11.00	12.00	0.25	0.50
tC_2N_3	16.00	14.75	8.25	9.50	0.55	0.25
iC_1N_1	30.50	36.00	5.25	4.50	1.00	1.25
iC_1N_2	33.75	46.25	6.00	3.50	0.25	2.00
iC_1N_3	36.25	48.25	4.00	3.25	1.25	1.25
iC_2N_1	46.25	50.75	4.00	2.25	1.50	2.25
iC_2N_2	48.25	54.00	3.50	2.00	1.50	2.00
iC_2N_3	49.50	58.50	3.25	1.75	1.25	2.25
Control	9.75	23.50	21.00	17.50	0.50	0.25
CD at 5%	5.15	6.55	2.52	0.75	0.72	0.45

d – dimethoate, q – quinalplhos, e – endosulfan, m – monocrotophos,
t – thiamethoxam, I – imidachlopid
C_1 – lower dose, C_2 – higher dose
N_1, N_2, N_3 – one, two and three rounds respectively

with dimethoate sprayed thrice. Also the highest panicle emergence was observed on those trees. Thus dimethoate, sprayed three times was found the most effective treatment. The least effective treatment was thiamethoxam in respect of nymphal mortality, mean number of galls and mean number of panicle emergence.

Singh *et al.* (1975) and Singh and Mishra (1978) successfully controlled the gall formation by spraying 0.04 per cent monocrotophos or 0.03 per cent dimethoate, three times at 10–12 days interval, after the honey dew and waxy excreta became visible on the egg spot, i.e., from 15th August to 15th September.

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Preliminary study on cause of gall formation in mango by *Apsylla cistellata* Buckton (Hemiptera: Psyllidae)

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ABSTRACT: Gall formation associated with the incidence of *Apsylla cistellata* in mango is attributed to the chemical changes in the host caused by feeding of *Apsylla* nymphs. The oils of infected and healthy plant parts were extracted using Clevenger's apparatus and Uv-vis spectra of samples were recorded ranging 200-300 nm in THF over Genesis 10 Thermospectronic USA. It was seen that there was significant change in the chemical composition of oil in the infected shoot when compared to healthy shoots in September while no change was seen in the infected shoot in April. Thus physiological effect caused by the feeding of *A. cistellata* on the mango trees is indicated to be associated with gall formation.

KEYWORDS: *Apsylla cistellata*, mango, UV-vis spectra

Mango (*Mangifera indica* L.), grown all over India is reported to be infested by more than 175 species of insects (Nayar *et al.*, 1976) *Apsylla cistellata* Buckton (Hemiptera: Psyllidae) is a serious pest of mango in plains of northern India, Nepal and Bangladesh. This insect causes the formation of hard cone shaped galls in place of buds, thus preventing flowering and fruit setting. This results in heavy yield loss and the varied aspects of this serious pest attack has been studied by different workers (Gupta and Joshi, 1985; Singh *et al.*, 1975; Srivastava *et al.*, 1982). Females lay eggs underside the leaf midrib in two parallel rows when the new flush is formed during March-April. Eggs hatch in the last week of August, synchronizing with the August flush of the mango trees. The freshly hatched nymphs remain stationary at the site of oviposition. The gall formation starts in small numbers by middle of September and become abundant by the last week of October. According to some workers the formation of galls may be due to environmental factors or some chemical changes due to feeding of *Apsylla* nymphs. The present investigation was undertaken to study whether the gall formation in mango is due to the chemical changes caused by feeding of *Apsylla* nymphs.

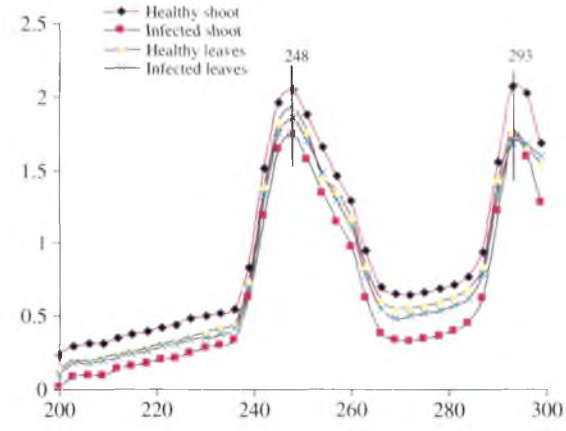

FIGURE 1. UV visible spectra of oil of healthy, tainted leaves and shoot, collected in the month of April.

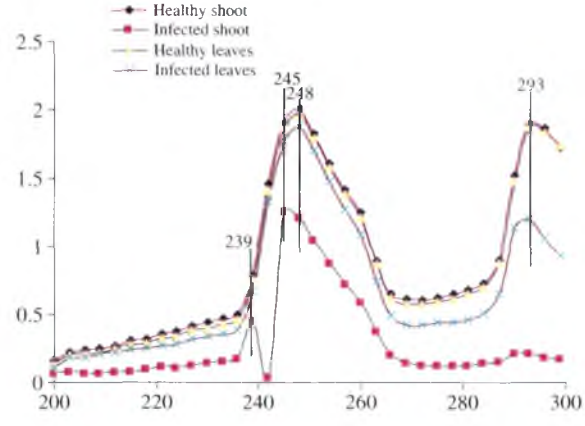

FIGURE 2. UV visible spectra of oil of healthy, infected leaves and shoot, collected in the month of November.

Heavily infested and healthy shoot of 20 cm length with the leaves were collected separately from 40–45 years old mango trees (cv. Dashehari). The samples were collected on 25 April 2007 and 15 August 2007, synchronising with flush emergence and hatching of egg, respectively. Both affected and normal shoot were finely chopped, dried immediately after sampling and kept in an oven at 50 °C for 48 h. The affected and normal leaves were kept in oven at 40 °C for 96 h. The dried samples were finely powdered in a grinder and the oil content was extracted by steam distillation using Clevenger's apparatus. A suitable amount of extracted oil was dissolved in known volume of THF. An aliquot of this solution was taken in cuvette and scanned from 200

to 300 nm on UV spectrophotometer (Genesis 10 Thermospectronic USA). Necessary dilution was made before taking the spectrum.

Effect of *A. cistellata* infestation on mango has clearly been observed in UV visible spectra of the oils extracted from infected shoot and leaves with reference to healthy leaves and shoot as control. All the samples collected in April showed a pair of absorption peak at 248 and 293 nm (Fig. 1). Among the samples collected during November, healthy shoot and healthy as well as infected leaves showed absorption peaks at 248 and 293 nm. But the oil from infected shoots collected in September showed two peaks at 239 and 245 nm (Fig. 2).

The results showed that there were no chemical changes in the infested part of mango during the month of April, when eggs were not hatched. But after hatching and when nymphs started feeding, some chemical changes occurred in infected shoots. The chemical changes were observed only in infected shoots and not in infected leaves. Prasad (1957) found that gall formation was the result of some secretions produced by the nymphs. Singh (1960) reported that the secretions produced by the eggs and nymphs brought about the swelling of the buds resulting in gall formation. He also suggested that the pressure exerted by hatching of eggs and irrigation caused by feeding nymphs might have brought about the hyperplasia of bud cells and swelling of buds, respectively, thus causing the galls. Singh *et al.* (1975) held that the fully developed embryos feeding at the oviposition sites injected some unknown chemicals which were responsible for gall formation. The changes in the absorption peaks of the oil of the infected shoots only in September and not in April indicate that the emerging nymphs causes significant physiological changes in the host and that is associated with the gall formation on the shoots.

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Oviposition deterrent property of certain plant extracts against rice leaf folder, *Cnaphalocrocis medinalis* (Guenee)

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ABSTRACT: Acetone and methanol extracts of the leaves of five plants *(Clerodendron viscosum, Amphineuron apulentum, Litsea citrata, Mimusops hexandra, Millettia auriculata)* were evaluated against *Cnaphalocrocis medinalis* (Guenee) as oviposition deterrent. In no-choice test, highest oviposition deterrence (80.99%) was recorded by methanol extract of *A. apulentum* and it was on par with all treatments except *C. viscosum* extract. In choice test, methanol extract of *Mimosops hexandra* with 98.15% oviposition deterrence came as the best. Acetone extract of *C. viscosum* and *A. apulentum* were inferior to neem oil.

KEYWORDS: *Cnaphalocrosis medinalis*, plant extract, oviposition deterrent

Plant products are being explored extensively for plant protection purpose with a view to reducing the hazards of chemical pesticides. In this context the oviposition deterrence of five locally available plants known for poisonous property were tested against the moths of rice leaf folder *Cnaphalacrocis medinalis* (Guenee) in the laboratory/net house.

Fresh plant materials (Table 1) collected from different parts of Nagaland were dried under shade, ground into powder and 30g of the powder was extracted separately using acetone and methanol. The extracts were filtered and used as stock solution. Neem oil and fenvalerate (20EC) were used as standards. Emulsions were prepared in water using Triton $\times$100 as emulsifier.

Insect culture was maintained in a net house by adopting the methods described by Waldbaner and Marciano (1979). Potted rice plants sprayed with respective emulsions to run off level were covered individually with nylon net and one pair of moths (male and female) was released in each replication (no-choice test). The treatments were distributed in Completely Randomized Design (CRD) with three replications for each treatment. In a second experiment, all the treated plants were enclosed in nylon net

TABLE 1. Plant extracts as oviposition deterrent against *C. medinalis*

Plant species	Extraction solvent	Concn. (%)	Reduction in oviposition (%)	
			No-choice test	Choice test
Clerodendrum viscosum[1]	Acetone	2	44.25 (41.73)	70.51 (8.41)
Amphineuron apulentum[2]	Acetone	2	76.83 (61.24)	77.02 (8.80)
A. apulentum	Methanol	2	80.99 (64.34)	87.45 (9.37)
Litsea citrata[3]	Acetone	2	76.53 (61.11)	92.19 (9.62)
L. citrata	Methanol	2	73.97 (59.45)	92.29 (9.63)
Mimusops hexandra [4]	Acetone	2	76.05 (61.09)	98.15 (9.93)
M. hexandra	Methanol	2	76.83 (59.58)	100 (10.02)
Millettia auriculata[5]	Acetone	2	69.80 (56.67)	87.81 (9.40)
M. auriculata	Methanol	2	73.49 (59.55)	96.29 (9.83)
Neem oil	—	1.05	72.07 (58.19)	93.26 (9.68)
Fenvalerate (20EC)	—	0.1%	67.42 (55.21)	84.93 (9.23)
Control	—	—	0 (0)	0 (0.7)
LSD (.05)	—	—	7.12	0.19

Figures in parentheses are arc sine transformed in no-choice test and square root transformed in choice test.
1, Verbenaceae; 2, Thelypteridaceae; 3, Lauraceae; 4, Sapotaceae; 5, Fabaceae

cage together. Treatments were replicated three times and were set up tin CRD. Moths (25 females and 20 males) were released and allowed to move freely among the treatments inside the net cover. Observation on number of eggs laid on leaves was recorded 48 h after the release of the moths. The percent reduction in egg laying over control was calculated by using the formula described by Patel and Goud (2003) and the data were statistically analyzed.

Under no-choice condition none of the treatments could completely prevent egg laying (Table 1). Maximum reduction (80.99%) in oviposition was recorded in plants treated with acetone and methanol extract of *A. apulentum* which was on par with neem oil (72.07%) and they came on par with the remaining treatments except for *C. viscosum* which was inferior.

When the moths were given choice for egg laying, methanol extract of *M. hexandra* (98.15%) also came on par; best treatment was significantly superior to neem oil. It was followed by acetone extract of *L. citrata*. Remaining treatments came on par and also on par with neem oil (93.26%). Minimum reduction (70.51%) in oviposition was seen in acetone extract of *C. viscosum* which was significantly higher compared with control.

Thus in both the situations (no-choice and choice condition) plant extracts reduced the egg laying of *C. medinalis* and the eggs were laid singly, not in distinct rows

as observed in untreated control. The oviposition deterrence of neem oil has been reported earlier also (Krishnaih and Kalode, 1991).

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Short Communication No. ent.33311

Encarsia flavoscutellum Zehntner (Hymenoptera: Aphelinidae) — A potential parasitoid of sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae) in North Karnataka

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ABSTRACT: An experiment was conducted to assess the parasitization by *Encarsia flavoscutellum* on *Ceratovacuna lanigera*. Among the different locations, significantly highest mean per cent parasitization was noticed at Sankeshwar (3.4%) whereas at Sameerwadi and Dharwad the per cent parasitization were 1.7 and 1.1 per cent, respectively, which were on par with each other. Among the locations and months, significantly higher per cent parasitization was recorded during November second fortnight in all the locations which were on par with each other.

KEYWORDS: *Encarsia flavoscutellum*, parasitoids, woolly aphid

The sugarcane crop yield is markedly influenced by many factors. Among them pests are known to cause considerable losses in cane yield as well as sugar output (David and Nandagopan, 1986; Anonymous, 2006). The sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae) is the latest threat to sugarcane crop as well as sugar industry in south India. In Karnataka, the infestation of this pest on sugarcane was first observed in Athani taluka of Belgaum district during September 2002 and subsequently it spread to other parts of the state.

Though synthetic insecticides are effective against *C. lanigera* the technology often fails due to extraneous factors. So biological control for its management is most ideal. The predators, viz., *Micromus igorotus* Banks, *Dipha aphidivora* Meyrick and *Eupeodes confracter* Weidmann are being employed to combat the pest. In addition to these predators, recently the establishment of *Encarsia flavoscutellum* Zehntner, a potential parasitoid of *C. lanigera* in and around Sankeshwar area was noticed. The

TABLE 1. Parasitization of sugarcane woolly aphid by *Encarsia flavoscutellum* at three locations of north Karnataka

Month	Per cent parasitization		
	Dharwad	Sankeshwar	Sameerwadi
September I[a]	0.00 (2.86)	1.80 (7.44)	0.00 (2.86)
September II	0.00 (2.86)	2.60 (9.18)	0.00 (2.86)
October I	0.00 (2.86)	3.60 (9.72)	0.00 (2.86)
October II	1.80 (7.37)	4.20 (11.77)	0.00 (2.86)
November I	2.60 (8.98)	4.80 (12.32)	1.80 (7.65)
November II	3.40 (9.99)	5.60 (13.29)	3.60 (10.82)
December I	2.00 (8.02)	7.60 (14.23)	4.40 (11.74)
December II	1.20 (5.91)	3.20 (9.97)	5.60 (13.25)
January I	0.00 (2.86)	1.40 (6.23)	1.80 (7.54)
January II	0.00 (2.86)	0.00 (2.86)	0.00 (2.86)
Mean	1.10 (5.46)	3.40 (9.75)	1.70 (6.53)
CD (Location)		2.01	
CD (Month)		2.46	
CD (Location X Month)		3.49	

Figures in the parentheses are arc sine transformed values.
[a] I, first fortnight; II, second fortnight.

present investigation was undertaken to know the influence of *E. flavoscutellum* in checking the pest population in north Karnataka.

The incidence of the parasitoid on *C. lanigera* was assessed in three different locations - Dharwad, Sankeshwar and Sameerwadi. Observations were made once in 15 days for five months, commencing from September 2006.

One hundred aphids were collected from five spots in each location to determine the extent of parasitism. Aphids were soaked in 70% alcohol for 10 min to free the body from woolly matter. Parasitised aphids were then counted under binocular microscope. The percentage of parasitization was calculated and the data were subjected to statistical analysis (two way ANOVA).

The results (Table 1) revealed that among the different locations, highest mean per cent parasitization was noticed at Sankeshwar (3.4%). At Sameerwadi and Dharwad the mean parasitization were 1.7 and 1.1 per cent, respectively, which were on par with each other. The parasitization recorded during the second fortnight of November (3.4%), first fortnight of December (7.6%), and second fortnight of December (5.6%) were the highest at Dharward, Sankeshwar and Sameerwadi, respectively. The percent parasitization in the two preceding fortnights came on par with the highest percentage. At Sankeshwar the incidence of the parasitoid was observed from the beginning of September to first fortnight of January while at Dharwad it was from October second fortnight to December second fortnight and at sameerwadi it was from November first fortnight to January first fortnight. Thus the parasitoid was present in 9, 5 and 5 out of the total 10 observations at Sankeshwar, Dharward and Sameerwadi, respectively. These variations might be due to the differences in crop stages, pest populations

and other ecological factors. Pramono *et al.* (1999) also reported that the per cent parasitization by *E. flavoscutellum* varied from 3.30 to 4.15 %. Similarly, Sharma *et al.* (2006) studied the activity of *E. flavoscutellum* and observed that it was prevalent from first fortnight of December 2004 to first fortnight of February 2005. The results showed that the parasitoid *E. flavoscutellum* is well established in the sugarcane belt of northern Karnataka at all the locations covered in the survey. In future this parasitoid can be expected to play a significant role in the suppression of sugarcane woolly aphid along with other natural factors.

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Record of a geometrid looper, *Ectropis* sp. infesting tea in Dooars tea plantations, West Bengal, India

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ABSTRACT: An unidentified species of *Ectropis* (Lepidoptera: Geometridae) is recorded infesting tea bushes in Dooars tea plantations, West Bangal, India

KEYWORDS: geometrid looper, *Ectropis* sp., tea, Dooars

Tea is the major plantation crop of Dooars, situated in the foot hills of sub-Himalayan West Bengal and is grown over a large area. A wide range of insects are active on tea bushes and many of them cause considerable crop loss every year. During 2006–2007, loopers of a geometrid moth, *Ectropis* were noticed in different tea gardens causing damage to the bushes. In 2006, the insect was found only in 3-4 estates of central Dooars, while in the early part of 2007 sudden outbreak of loopers (including *Ectropis*) occurred in some of the tea sub-districts of Dooars. The genus *Ectropis* includes several species of which only two were reported as tea pests in different parts of the world. Among them, *Ecropis obliqua* Prout is known to infest tea in east Asia for a long time and it is considered as one of the major pests of tea there (Chen and Huang, 2001). In Sri Lanka, the twig caterpillar, *Ectropis bhurmitra* (Wkr) is known to occur for years but has never been a serious pest until a major outbreak occurred during 1963–65 (Danthanarayana and Kathiravetpillai, 1969).

Ectropis is not seen included as a pest of tea in Dooars in any early publication on tea pests (Watt and Mann, 1903; Antram, 1911; Das, 1965) nor could any reference of it be found in any of the publications of Tea Research Association so far.

The early instar looper is deep brownish with shades of black and cream. The body is cylindrical. Mature larva is rusty brown with creamy to baize shades on head and latter part of body. Two lateral black bands are present above the reddish spiracular zone. In some loopers, one black patch is noticed on the dorsal abdominal segment. Full grown loopers measure 28–35 mm. The adult wing base is cream to baize in colour, with wavy lines of black, ash or even white. The fasciae are mostly strongly crenulate and/or nearer the margin, interrupted at the veins. The under side of the

wings is creamy with fainter spots. Wing expense of male is 32–39 mm and of female, 45–50 mm.

This insect is polyphagous and besides tea it was found feeding on the leaves of *Bauhinia variegata, Terminalia tomentosa, Delonix regia, Albizzia* sp., *Derris robusta, Cassia* sp. and *Indigofera teysmanii.*

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GUIDELINES TO AUTHORS

Scope: ENTOMON will publish original research papers on insects, arachnids and myriapods. Reviews are not acceptable.

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Nair M. R. G. K. and Mohandas N. (1962) On the biology and control of *Carvalhoeia arecae*, a pest of areca palms in Kerala. Indian Journal of Entomology 24: (1) 86–93.

Jalaja M. Muraleedharan D. and Prabhu V. K. K. (1973) Effect of extirpation of median neurosecretory cells on reproduction in the female red cotton bug, *Dysdercus cingulatus*. Journal of Insect Physiology 19(1): 29–36

Books and Articles in Books:

Novak V. J. A. (1966) *Insect Hormones*. Methuen and Co., 478 pp.

Wigglesworth V. B. (1964) The hormonal regulation of growth and reproduction in insects. In: *Advances in Insect Physiology* Vol. 2 (Eds. Beament J. W. L., Treherne J. E. and Wigglesworth V. B), Academic Press, London, pp 247–335.

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